

FORM PTO-1390  
(REV. 6-87)U.S. DEPARTMENT OF COMMERCE  
PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER

29 SEP 1998  
1102-98TRANSMITTAL LETTER TO THE UNITED STATES  
DESIGNATED/ELECTED OFFICE (DO/EO/US)

09/155514

INTERNATIONAL APPLICATION NO.  
PCT/JP98/00370INTERNATIONAL FILING DATE  
29 January 1998 (29.01.98)PRIORITY DATE CLAIMED  
29 January 1997 (29.01.97)TITLE OF INVENTION  
CHIMERIC PROTEINS, THEIR HETERODIMER COMPLEXES, AND PLATELET SUBSTITUTESAPPLICANT(S) FOR DO/EO/US  
Mie Kainoh and Toshiaki Tanaka

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items under 35 U.S.C. 371:

1. ■ This express request to immediately begin national examination procedures (35 U.S.C. 371(f)).
2. ■ The U.S. National Fee (35 U.S.C. 371(c)(1)) and other fees as follows:

CLAIMS	(1) FOR	(2) NUMBER FILED	(3) NUMBER EXTRA	(4) RATE	(5) CALCULATIONS
TOTAL CLAIMS		64 -20=	44	x \$22.00	\$ 968.00
INDEPENDENT CLAIMS		3 -3=	0	x \$82.00	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)				+ \$270.00	270.00
BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(4)):					
<input type="checkbox"/> International preliminary examination fee paid to USPTO (37 CFR 1.482).....					\$720.00
<input type="checkbox"/> No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)).....					\$790.00
<input type="checkbox"/> Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO.....					\$1,070.00
<input type="checkbox"/> International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2) to (4).....					\$ 98.00
■ International Search Report will be forwarded from WIPO.....					\$930.00
Surcharge of \$_____ for furnishing the National fee or oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 mos. from the earliest claimed priority date (37 CFR 1.482(e)).					\$130.00
TOTAL OF ABOVE CALCULATIONS					=2,168.00
Reduction by 1/2 for filing by small entity, if applicable. Affidavits must be filed also. (Note 37 CFR 1.9, 1.27, 1.28.)					
SUBTOTAL					+2,168.00
Processing fee of \$_____ for furnishing the English Translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 mos. from the earliest claimed priority date (37 CFR 1.482(f)).					\$130.00
TOTAL NATIONAL FEE					\$2,168.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)).					\$40.00
TOTAL FEES ENCLOSED					\$2,168.00

- A check in the amount of \$2,168.00 to cover the above fees is enclosed.
- ☐ Please charge my Deposit Account No. 13-3405 in the amount of \$\_\_\_\_\_ to cover the above fees.  
A duplicate copy of this sheet is enclosed.
- The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 13-3405. A duplicate copy of this sheet is enclosed.

3. A copy of the International Application as filed (35 U.S.C. 371(c)(2))
- ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
  - ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
  - ☒ has been transmitted by the International Bureau.
4. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
5. Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
- ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
  - ☐ have been transmitted by the International Bureau.
6. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
7. ☐ An oath or declaration of the inventor (35 U.S.C. 371(c)(4)).
8. ☐ A translation of the Annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Other document(s) or information included:

9. ☐ An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98.
10. ☐ An Assignment document for recording and a Recordation Form Cover Sheet - Patents Only. Please mail the recorded assignment document to the person whose signature, name and address appears at the bottom of this page.
11. The above checked items are being transmitted
- ☐ before the 18th month publication.
  - ☒ after publication and the Article 20 communication but before 20 months from the priority date.
  - ☐ after 20 months but before 22 months (surcharge and/or processing fee included).
  - ☐ after 22 months (surcharge and/or processing fee included).  
*Note: Petition to revive (37 C.F.R. 1.137(a) or (b)) is necessary if 35 U.S.C. 371 requirements submitted after 22 months and no proper demand for International Preliminary Examination was made by 19 months from the earliest claimed priority date.*
  - ☐ by 30 months and a proper demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
  - ☐ after 30 months but before 32 months and a proper demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date (surcharge and/or processing fee included).
  - ☐ after 32 months (surcharge and/or processing fee included).  
*Note: Petition to revive (37 C.F.R. 1.137(a) or (b)) is necessary if 35 U.S.C. 371 requirements submitted after 32 months and a proper demand for International Preliminary Examination was made by 19 months from the earliest claimed priority date.*
12. At the time of transmittal, the time limit for amending claims under Article 19
- ☐ has expired and no amendments were made.
  - ☐ has not yet expired.
13. ☐ Certain requirements under 35 U.S.C. 371 were previously submitted by the applicant on \_\_\_\_\_, namely:

SCHNADER HARRISON SEGAL & LEWIS

Date: 29 SEPT 1998

By: T. Phil Ch... 31750  
Austin R. Miller, Reg. No. 16,602  
36th Floor  
1600 Market Street  
Philadelphia, PA 19103  
(215) 563-1810

3/PRTS

Express Mail Label EL139842061US

09/155514

405 Rec'd PCT/PTO 28 SEP 1998

21 SEP 1998 29 SEP 1998

# SPECIFICATION

Chimeric proteins, their heterodimer complexes, and platelet substitutes

## Technical Field:

The present invention relates to chimeric proteins consisting of an integrin and an immunoglobulin, their heterodimer complexes, a production process thereof, their applications as drugs and reagents, etc. Furthermore, the present invention relates to medicinal application of isolated extracellular matrix receptors such as integrin-immunoglobulin chimeric protein heterodimer complexes, as platelet substitutes.

## Background Arts:

Various cells have receptors which mediate the adhesion between a cell and a cell and receptors which mediate the adhesion between a cell and an extracellular matrix, and these receptors play important roles in immune reaction, inflammatory reaction, development, morphogenesis, wound healing, hemostasis, cancerous metastasis, etc. By separating and identifying the receptors which participate in these phenomena, the existence of so-called cell adhesion molecules has been clarified. Many of the molecules identified one after another are classified in reference to their structural features into integrin superfamily, immunoglobulin superfamily, selectin family, cadherin family, etc. (Corliss,

RECEIVED  
JUN 11 1994

T. M. and Harlan, J. M., Blood, 84, 2068-2101 (1994)). Of these families, the immunoglobulin superfamily, selectin family and cadherin family mediate mainly the adhesion between a cell and a cell, while the integrin superfamily is the so-called extracellular matrix receptors which mediate the adhesion to extracellular matrices such as fibronectin and collagens. In addition, extracellular matrix receptors which do not belong to any of these adhesion molecule families include CD26 (DDP1V), CD44, GPIV, GPVI, GPIb-vWF, etc. CD26 is a receptor for collagens, and CD44 is a receptor for hyaluronic acid, fibronectin and collagens ("Adhesion Molecules" p. 32-42, Masayuki Miyasaka (1991), Medical View (in Japanese)). Furthermore, it is reported that among the membrane glycoproteins (GPs) existing on platelets, GPIV, GPVI, GPIb-vWF, etc. are also collagen receptors ("Platelet Receptors", p. 119-132, Minoru Ohkuma et al., (1992), Kinpodo (in Japanese)).

A receptor belonging to the integrin superfamily has a heterodimer complex structure in which two subunits,  $\alpha$ -chain and  $\beta$ -chain as mutually different membrane proteins are associated with each other non-covalently (Hynes, R. O., Cell, 48, 549-554 (1987)). In the past, the integrin superfamily was classified into three subfamilies;  $\beta$ 1 integrin,  $\beta$ 2 integrin and  $\beta$ 3 integrin. Later, new  $\beta$  chains and  $\alpha$  chains were discovered one after another, and presently eight  $\beta$

chains ( $\beta 1, \beta 2, \beta 3, \beta 4, \beta 5, \beta 6, \beta 7$  and  $\beta 8$  and fifteen  $\alpha$  chains ( $\alpha 1, \alpha 2, \alpha 3, \alpha 4, \alpha 5, \alpha 6, \alpha 7, \alpha 8, \alpha 9, \alpha v, \alpha L, \alpha M, \alpha X, \alpha 11b$  and  $\alpha E$ ) have been identified (Elner, S. G. and Elner, V. M. *Inv. Ophthal. Vis. Sci.*, 37, 696-701 (1996)). It is known that each  $\beta$  chain is associated with one to eight  $\alpha$  chains, and as a result, 21 pairs of an  $\alpha$  chain and a  $\beta$  chain, i.e., integrin molecules have been identified (Elner, S. G. and Elner, V. M., *Inv. Ophthal. Vis. Sci.*, 37, 696-701 (1996)). They include  $\alpha 4 \beta 1$  (VLA-4,  $\beta 1$  integrin),  $\alpha L \beta 2$  (LFA-1,  $\beta 2$  integrin),  $\alpha M \beta 2$  (Mac-1,  $\beta 2$  integrin),  $\alpha 11b \beta 3$  (GP11b/111a,  $\beta 3$  integrin), etc. now targeted for drug development (*Drug and Market Development*, 6, 201-205 (1995)). Many other integrins are also expected to have relations with diseases.

The heterodimer complex structure of an integrin plays an important role in binding to a ligand (Hynes, R. O., *Cell*, 48, 549-554 (1987)). For example, it is estimated that the ligand binding region on an integrin consists of both an  $\alpha$  chain and a  $\beta$  chain (Hynes, R. O., *Cell*, 69, 11-25 (1992)). The fact that integrins having the same  $\alpha$  chain but associated with a different  $\beta$  chain, or integrins having the same  $\beta$  chain but associated with a different  $\alpha$  chain are respectively different in substrate specificity (Elner, S. G. and Elner, V. M., *Inv. Ophthal. Vis. Sci.* 37, 696-701 (1996)) supports this assumption. On the other hand, it was reported that the  $\alpha$  chains of some integrins have an sequence called an I domain

consisting of about 180 amino acids inserted in the molecule, and data suggest that the I domain only could be bound to a ligand were reported (Ueda, T. et al., Proc. Natl. Acad. Sci. USA, 91, 10680-10684 (1994)). However, it was also reported that the I domain of an  $\alpha$  domain and the integrin as its original heterodimer complex are different in the style of binding to a ligand (Kamata, T. and Takada, Y., J. Biol. Chem., 269, 26006-26010 (1994)). It is also not clarified yet whether such parameters as specificity and affinity to a ligand are identical. It is not reported that in the case of an integrin not containing the I domain, for example, in the case of  $\alpha 4 \beta 1$  a partial structure only is bound to a ligand.

If any integrin isolated and prepared retains its heterodimer complex structure, hence the ligand binding capability, it can be used for studying the style of binding to a ligand in a state close to nature. Furthermore, it can be used as it is as a drug and can also be used as a reagent for measuring the amount of a ligand in tissue or serum or as a material for searching for adhesion inhibiting compounds very usefully. However, isolating and preparing an integrin with its function retained is said to be very difficult. One reason is that since the association between an  $\alpha$  chain and a  $\beta$  chain of an integrin is maintained non-covalently as described before, they are easily dissociated during isolation and preparation. Since an integrin is a membrane protein, the

necessity of using a surfactant, etc. for solubilization is considered to be a large cause in the dissociation of the complex. In other words, the non-covalent preservation of functional structure inhibits the preparation of such an integrin.

In spite of the difficulty as described above, some cases were reported, in which an integrin heterodimer complex was isolated and prepared with its function retained. For cases of  $\alpha 2 \beta 1$ ,  $\alpha 5 \beta 1$  and  $\alpha v \beta 3$ , it was reported that the binding to a ligand can be determined by letting a liposome incorporate an integrin purified by using affinity column chromatography (Santoro, S. A. et al., Biochem. Biophys. Res. Comm., 153, 217-223 (1988), Pytela, R. et al. Cell, 40, 191-198 (1985), Pytela, R. et al., Method Enzymol., 144, 475-489 (1987)). For other cases, it was that if purified  $\alpha 5 \beta 1$  or  $\alpha v \beta 3$  is coated on a plate, a peptide which inhibits the cell adhesion through the integrin can be selected (Koivunen, E. et al., J. Biol. Chem., 268, 20205-20210 (1993), Healy, J. M. et al., Biochemistry, 34, 3948-3955 (1995)). For further other cases, it was reported that if purified  $\alpha v \beta 3$  or  $\alpha 4 \beta 1$  is coated on a plate, the binding to a ligand can be determined (Charo, I. F. et al., J. Cell Biol., 111, 2795-2800 (1990), Makarewicz, R. et al., J. Biol. Chem., 269, 4005-4011 (1994), Paul Mould, A. et al., J. Biol. Chem., 269, 27224-27230 (1994)). For a still further other case, it was reported that if an extracellular

portion of  $\alpha 11b\beta 3$  heterodimer complex prepared by gene manipulation is coated on a plate through a complex specific antibody, the binding to a ligand can be determined (Gulino, D. et al., Eur. J. Biochem., 227, 108-115 (1995)). These cases suggest that to exert the function of a purified integrin, its heterodimer complex must be bound to or included in any carrier. The reason why a carrier is considered to be necessary is that since a heterodimer complex is associated non-covalently in a solution, it tends to be dissociated and as a result, cannot retain its functional structure. In the finally stated case, only a molecule with a heterodimer complex structure is selected using a complex specific antibody, in a design to determine the binding in a state where both the chains are not dissociated from each other.

As a case requiring no carrier, it was reported that purified  $\alpha 1\beta 1$ , or  $\alpha 2\beta 1$  allows the determination of the bonding to a ligand dependent on high concentration of metal ions even without using any carrier (Pfaff, M. et al., Eur. J. Biochem., 225, 975-984 (1994)). In this case, the surfactant added in the process of purification plays a role similar to that of a liposome, acting as a carrier. For a further other case, it was reported that an extracellular of  $\alpha M\beta 2$  heterodimer complex prepared by using gene manipulation is bound to a ligand (Berman, P. W. et al., J. Cell Biochem., 52, 183-195 (1993)). These cases do not suggest the necessity of

any carrier as described before, but the disadvantage that the association of molecules in a heterodimer complex is retained non-covalently is not improved.

As a still further other case, a chimeric protein consisting of  $\alpha$ d and an immunoglobulin is disclosed (Japanese Patent Laid-Open (Kokai) No. 8-507933), but only the result of immune precipitation is reported, without examining the binding to a ligand. Furthermore, since a  $\beta$  chain is not expressed in the chimeric protein as an immunoglobulin, the binding between an  $\alpha$  chain and a  $\beta$  chain remains non-covalent.

The above facts suggest that any integrin with an  $\alpha$  chain and a  $\beta$  chain structurally stably associated and with its function retained has never been successfully prepared. That a complex structure is unstable restricts the use of its molecule.

Of the molecules belonging to the integrin superfamily, integrin  $\alpha 2 \beta 1$  is an extracellular matrix receptor found to be expressed in T cells, platelets, etc. activated for long time. However, it was reported that the  $\alpha 2 \beta 1$  on the cell surfaces of platelets and fibroblasts is bound to collagens only and that the  $\alpha 2 \beta 1$  on the surfaces of vascular endothelial cells is bound to both collagens and laminins (Elices, M. J. et al., Proc. Natl. Acad. Sci. USA, 86, 9906-9910 (1989)), and it is speculated that the function of  $\alpha 2 \beta 1$  becomes different,

depending on cells.

In relation to the conditions of diseases, there are reports to suggest that integrin  $\alpha 2 \beta 1$  plays an important role for wound healing and cancerous metastasis (Shiro, J. A. et al., Cell, 67, 403-410 (1991), Chen, F. et al., J. Exp. Med., 173, 1111-1119 (1991), Chan, B. M. C. et al., Science, 251, 1600-1602 (1991)). Furthermore, it was reported that from the analysis of platelet function of patients with bleeding tendency, the adherence of platelets and collagens through integrin  $\alpha 2 \beta 1$  has close relation with the first step of hemostasis and thrombosis process (Nieuwenhuis, H. K. et al., Nature, 318, 470-472 (1985)). Though the relations of integrin  $\alpha 2 \beta 1$  with conditions of diseases are suggested like this, any medical application of using the integrin  $\alpha 2 \beta 1$  protein and other isolated extracellular matrix receptor proteins under physiological ion condition or in the presence of plasma components has not been examined.

On the other hand, the necessity for artificial substitutes of platelets used as blood preparations in the clinical field is growing, and various attempts have been reported (Progress of Medicine 179, 406-407 (1996), Clinical Blood 37, 1353-1361 (1997) (respectively in Japanese)). However, they are not yet practically available.

Disclosure of the Invention:

The present invention relates to chimeric proteins in

which the  $\alpha$  chain and  $\beta$  chain of an integrin are combined with the heavy chain or light chain of an immunoglobulin, their heterodimer complexes, a production process thereof, a method for testing the binding of an integrin-immunoglobulin chimeric protein heterodimer complex to a ligand and a cell, substances bound to an integrin obtained by using the method, a method for searching for a substance inhibiting the binding between an integrin and a ligand using the integrin-immunoglobulin chimeric protein heterodimer complex, substances for inhibiting the binding, and the application of integrin-immunoglobulin chimeric protein heterodimer complexes as drugs and reagents. Furthermore, the present invention relates to platelet substitutes containing an integrin-immunoglobulin chimeric protein heterodimer complex or any other isolated extracellular matrix receptor as an active ingredient.

#### Brief Description of the Drawings:

Fig. 1 shows that  $\alpha 4 \cdot \text{IgG}$  heavy chain- $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein heterodimer complex is bound to VCAM-1 expressing cell, and that the binding is inhibited by an anti-integrin antibody or EDTA, a cationic chelating agent.

Fig. 2 shows that  $\alpha 4 \cdot \text{IgG}$  heavy chain- $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein heterodimer complex is bound to CS-1 peptide, and that the binding is inhibited by an anti-integrin antibody or EDTA, a cationic chelating agent.

Fig. 3 shows that the binding between  $\alpha 4 \cdot \text{IgG}$  heavy

chain- $\beta$ 1IgG heavy chain chimeric protein heterodimer complex and CS-1 peptide is inhibited by GPEILDVPST, and is not inhibited by any other peptide.

Fig. 4 shows that  $\alpha$ 2-IgG heavy chain- $\beta$ 1IgG heavy chain chimeric protein heterodimer complex is bound to a collagen, and that the binding is inhibited by an anti-integrin antibody and EDTA, a cationic chelating agent.

Fig. 5 shows that  $\alpha$ 2-IgG heavy chain- $\beta$ 1IgG heavy chain chimeric protein heterodimer complex liposome is bound to a collagen in the presence of plasma.

Fig. 6 shows that the binding of  $\alpha$ 2-IgG heavy chain- $\beta$ 1IgG heavy chain chimeric protein heterodimer complex liposome to a collagen is inhibited by an anti-integrin antibody or EDTA, a cationic chelating agent.

#### The Best Embodiments of the Invention:

The extracellular matrix receptors in the present invention refer generally to the receptors which mediate the adhesion between a cell and an extracellular matrix. The receptors include the integrin superfamily having a heterodimer complex structure in which an  $\alpha$  chain and a  $\beta$  chain are non-covalently associated with each other as two membrane proteins (Corliss, T. M. and Harlan, J. M. Blood, 84, 2068-2101 (1994)), and other receptors such as CD26 (DDP1V), CD44, GPIV, GPVI, GPb-vWF, etc. The integrins in the present invention refer to molecules belonging to the integrin

superfamily, and also include the isomers of the molecules belonging to the family. The  $\alpha$  chains of the present invention include 15  $\alpha$  chains, i.e.,  $\alpha 1, \alpha 2, \alpha 3, \alpha 4, \alpha 5, \alpha 6, \alpha 7, \alpha 8, \alpha 9, \alpha v, \alpha L, \alpha M, \alpha X, \alpha 11b$  and  $\alpha E$ , and among them,  $\alpha 4$  and  $\alpha 2$  are preferable, though preferable  $\alpha$  chains are not limited to them. The  $\beta$  chains of the present invention include eight  $\beta$  chains, i.e.,  $\beta 1, \beta 2, \beta 3, \beta 4, \beta 5, \beta 6, \beta 7$  and  $\beta 8$  and among them,  $\beta 1$  is preferable, though preferable  $\beta$  chains are not limited to it. The integrin molecules as pairs respectively consisting of an  $\alpha$  chain and a  $\beta$  chain include the twenty one integrins stated in Elner, S. G. and Elner, V. M., *Inv. Ophthal. Vis. Sci.*, 37, 696-701 (1996), though not limited to them.

A chimeric protein consisting of the  $\alpha$  chain of an integrin and the heavy chain or light chain of an immunoglobulin refers to a molecule in which the extracellular region of the  $\alpha$  chain of an integrin is bound to the constant region of the heavy chain or light chain contained an immunoglobulin. In this case, a chimeric protein in which N terminus side of the protein is integrin molecule and then connected to an immunoglobulin molecule side by side is preferable. A chimeric protein consisting of the  $\beta$  chain of an integrin and the heavy chain or light chain of an immunoglobulin refers to a molecule in which the extracellular region of the  $\beta$  chain of an integrin is bound to the constant

region of the heavy chain or light chain contained in an immunoglobulin. Also in this case, a chimeric protein in which N terminus side of the protein is an integrin molecule and then connected to an immunoglobulin molecule side by side is preferable. In either case of  $\alpha$  chain or  $\beta$  chain, a chimeric protein bound to the heavy chain of an immunoglobulin is preferable.

The isotype of the immunoglobulin to be bound to the  $\alpha$  chain or  $\beta$  chain is not especially limited. Any of IgG, IgM, IgA and IgE can be used, but it is preferable to use IgG. The subclasses of IgG include IgG<sub>1</sub>, IgG<sub>2</sub>, IgG<sub>3</sub> and IgG<sub>4</sub>, but it is preferable to use IgG<sub>1</sub>. Furthermore, it is possible to use a molecule with a dimer structure having a disulfide bond between molecules instead of the immunoglobulin.

In the present invention, a molecule in which a chimeric protein consisting of the  $\alpha$  chain of an integrin and the heavy chain or light chain of an immunoglobulin and a chimeric protein consisting of the  $\beta$  chain of the integrin and the heavy chain or light chain of the immunoglobulin are associated with each other is called an integrin-immunoglobulin chimeric protein heterodimer complex. In this case, a combination consisting of  $\alpha$  chain-immunoglobulin heavy chain (which means a chimeric protein consisting of an  $\alpha$  chain and the heavy chain of an immunoglobulin; hereinafter this applies) and  $\beta$  chain-immunoglobulin heavy chain, a combination consisting of

$\alpha$  chain-immunoglobulin heavy chain and  $\beta$  chain-immunoglobulin light chain, and a combination consisting of  $\alpha$  chain-immunoglobulin light chain and  $\beta$  chain-immunoglobulin heavy chain are preferable. A combination consisting of  $\alpha$  chain-immunoglobulin heavy chain and  $\beta$  chain-immunoglobulin heavy chain is more preferable.

In the integrin-immunoglobulin chimeric protein heterodimer complex of the present invention, the  $\alpha$  chain can be  $\alpha 1, \alpha 2, \alpha 3, \alpha 4, \alpha 5, \alpha 6, \alpha 7, \alpha 8, \alpha 9, \alpha v, \alpha L, \alpha M, \alpha X, \alpha 11b$  or  $\alpha E$ , and the  $\beta$  chain can be  $\beta 1, \beta 2, \beta 3, \beta 4, \beta 5, \beta 6, \beta 7$  or  $\beta 8$ . It is preferable that the  $\alpha$  chain is  $\alpha 4$  or  $\alpha 2$  and that the  $\beta$  chain is  $\beta 1$ , though preferable chains are not limited to them.

The process for preparing an integrin-immunoglobulin chimeric protein heterodimer complex is described below, but the process is not limited thereto.

A DNA coding for the  $\alpha$  chain and  $\beta$  chain of an integrin can be obtained using the information of known cDNA sequences by such a method as gene amplification based on the PCR method, cDNA cloning or DNA synthesis. For example, the DNA sequences of  $\alpha 4$  and  $\beta 1$  are already reported in literature (Takada, Y. et al., EMBO J., 8, 1361-1368 (1989), Scott Argraves, W. et al., J. Cell Biol., 105, 1183-1190 (1987)). A DNA coding for the  $\alpha$  chain and  $\beta$  chain of an integrin can also be obtained by the expression cloning using an antibody, etc. For binding to a DNA coding for the constant region of

an immunoglobulin, it is desirable to take out a DNA coding for the extracellular portions only of the  $\alpha$  chain and  $\beta$  chain of an integrin. For this purpose, it is preferable to use the PCR method and DNA synthesis. The extracellular portion of either an  $\alpha$  chain or  $\beta$  chain refers to the polypeptide sequence on the N terminus side from the portion speculated to be the transmembrane portion. Its partial sequence can also be used as far as the ligand binding capability is retained, but it is preferable to use most of the portion considered to be an extracellular region. For taking out a DNA, it is necessary to adjust for adaptation of frames after linking to a DNA coding for an immunoglobulin. For example, this can be achieved by modifying the primer when a DNA fragment is taken out by the PCR method. In this case, it is desirable to design for ensuring that amino acid modification is not caused by the base substitution of the primer. However, amino acid substitution is allowed as far as the function of the chimeric protein is not changed. For obtaining a DNA by chemical synthesis, the purpose can be achieved by designing a sequence to ensure the linking to a DNA coding for an immunoglobulin. In the case of cDNA, a DNA capable of being bound to a DNA coding for an immunoglobulin can be prepared by using DNA fragmentation and a synthetic DNA.

Then, a DNA coding for an immunoglobulin is prepared. In

the present invention, it is desirable to use DNAs coding for the heavy chain and light chain of a human immunoglobulin, but DNAs coding for an immunoglobulin of another animal species can also be used. The preparation of a DNA coding for human IgG is already reported (Ellison, J. W. et al., Nucleic Acids Res., 10, 4071-4079 (1982)), but the preparation is not limited to this method. Any method similar to the above mentioned method for preparing DNAs coding for the  $\alpha$  chain and  $\beta$  chain of an integrin can also be used. In the present invention, for the heavy chain of a human immunoglobulin, it is preferable to use a genomic DNA, but a cDNA can also be used. As the DNA for the heavy chain of a human immunoglobulin, it is preferable to use a portion coding for the hinge region, CH2 region or CH3 region, but a DNA coding for the entire constant region of CH1 - CH3 can also be used. For the light chain of an immunoglobulin, a DNA coding for the CL region is used. Finally, a DNA coding for the extracellular portion of an  $\alpha$  chain or  $\beta$  chain and a DNA coding for the constant region of human immunoglobulin heavy chain are linked with in frame. The obtained DNA codes for a polypeptide starting from the methionine of translation initiation and having the signal sequence of the  $\alpha$  chain or  $\beta$  chain of an integrin, its extracellular region and the constant region of human immunoglobulin heavy chain linked in this order.

The DNA coding for a chimeric protein consisting of the  $\alpha$  chain of an integrin and the heavy chain or light chain of an immunoglobulin, or the DNA coding for a chimeric protein consisting of the  $\beta$  chain of an integrin and the heavy chain or light chain of an immunoglobulin respectively obtained in the above is functionally linked in a proper expression control sequence, to obtain a recombinant vector. The general methods concerning gene recombination such as the method for preparing the recombinant vector, the method for transfecting it into a cell are described in a published book ("Molecular Cloning", Sambrook et al., (1989) Cold Spring Harbor Lab. Press, New York), but the methods are not limited to those stated there. In the present invention, it is desirable to use an expression control sequence suitable for protein expression in animal cells. For example, for manifestation of insect cells, polyhedrin promotor, p10 promotor, etc. are generally used as expression control sequences, and for expression of other animals' cells, SR $\alpha$  promotor, cytomegalovirus derived promotor, simian virus 40 derived promotor, polyhedrin promotor, p10 promotor, etc. are used. However, the expression control sequences are not limited to them. In the present invention, it is preferable to use SR $\alpha$  promotor.

If the obtained recombinant vector is transfected into a cell, a cell capable of producing an integrin-immunoglobulin

chimeric protein heterodimer complex can be obtained. In this case it is preferable to use an animal derived cell as a host. For example, COS cell (simian renal cell), CHO cell (Chinese Hamster ovarian cell), Sf9 (insect cell), etc. are generally used as hosts. Furthermore, myeloma cells such as P3U1 and Y3 can also be used. Other established cell lines and cloned cells can also be used, but the cells used as hosts are not limited to them. In the present invention, it is preferable to use a CHO cell.

It is known that the methods for transfecting a recombinant vector into a cell include the lipofectin method, calcium phosphate method, electroporation method, etc., and any of the methods can be used. The method is not limited to them. It is preferable that when a cell is transfected by using a recombinant vector, a recombinant vector for expression of a chimeric protein consisting of the  $\alpha$  chain of an integrin and the heavy chain or light chain of an immunoglobulin and a recombinant vector for expression of a chimeric protein consisting of the  $\beta$  chain of the integrin and the heavy chain or light chain of the immunoglobulin are transfected into the cell one after another using different drug resistance markers. The recombinant vectors can be transfected in any order or simultaneously. It is desirable that the two recombinant vectors to be transfected are vectors for expression of a combination consisting of  $\alpha$  chain.

SECRET

immunoglobulin heavy chain (which means a chimeric protein consisting of an  $\alpha$  chain and the heavy chain of an immunoglobulin; hereinafter this applies) and  $\beta$  chain-immunoglobulin heavy chain, or  $\alpha$  chain-immunoglobulin heavy chain and  $\beta$  chain-immunoglobulin light chain, or  $\alpha$  chain-immunoglobulin light chain and  $\beta$  chain-immunoglobulin heavy chain. Any of these combinations can be adopted, but a combination of recombinant vectors for expression of  $\alpha$  chain-immunoglobulin heavy chain and  $\beta$  chain-immunoglobulin heavy chain is desirable.

In any transfection method and any combination of vectors, it is important to select a cell which is transfected by the two recombinant vectors and produces a chimera protein consisting of an  $\alpha$  chain and the heavy chain or light chain of an immunoglobulin and a chimera protein consisting of a  $\beta$  chain and the heavy chain or light chain of an immunoglobulin simultaneously almost by the same amounts. This can be achieved by measuring the amounts of the chimeric protein consisting of an  $\alpha$  chain and the heavy chain or light chain of an immunoglobulin and a chimeric protein consisting of a  $\beta$  chain and the heavy chain or light chain of an immunoglobulin produced in the cultured supernatant solution of the cell transfected by the recombinant vectors. For measurement, for example, the transfected cell can be cultured in a medium containing  $^{35}\text{S}$  according to any publicly known method, for

09165514-111798

labeling the proteins, and the amounts of the chimeric protein consisting of an  $\alpha$  chain and the heavy chain or light chain of an immunoglobulin and a chimeric protein consisting of a  $\beta$  chain and the heavy chain or light chain of an immunoglobulin existing in the cultured supernatant solution can be estimated by immunoprecipitation using an anti- $\alpha$  chain antibody or an anti- $\beta$  chain antibody respectively. As another method, the amounts the chimeric protein consisting of an  $\alpha$  chain and the heavy chain or light chain of an immunoglobulin and a chimeric protein consisting of a  $\beta$  chain and the heavy chain or light chain of an immunoglobulin existing in the cultured supernatant solution can be estimated according to the ELISA method using an anti-human immunoglobulin antibody and an anti- $\alpha$  chain antibody or an anti- $\beta$  chain antibody. Anyway, it is preferable to select a clone which produces almost the same large amounts of the chimeric proteins of the  $\alpha$  and  $\beta$  chains in the culture supernatant, for preparing an integrin-immunoglobulin chimeric protein heterodimer complex. The methods for labeling proteins, the methods of immunoprecipitation and the general methods of ELISA are described in a published book ("Antibody" Harlow, E., and Lane, D. (1988), Cold Spring Harbor Lab. Press, New York), but the methods are not limited to them. Any other method can also be used for detecting chimeric proteins.

The obtained transfected cell can be cultured according to

a general cell culture method, to produce an integrin-immunoglobulin chimeric protein heterodimer complex. It is preferable that the medium contains about 5% of serum of a low immunoglobulin concentration, but any generally known serum-containing medium or a serum-less medium can also be used. After completion of cell culture, the cells and solid matter are removed by such operation as centrifugation, and the culture supernatant containing an integrin-immunoglobulin chimeric protein heterodimer complex is collected.

It can be estimated that the cultured supernatant solution contains not only the integrin-immunoglobulin chimeric proteins in which the chimeric protein consisting of an  $\alpha$  chain and the heavy chain or light chain of an immunoglobulin and a chimeric protein consisting of a  $\beta$  chain and the heavy chain or light chain of an immunoglobulin form a heterodimer complex, but also the chimeric protein consisting of an  $\alpha$  chain and the heavy chain or light chain of an immunoglobulin and a chimeric protein consisting of a  $\beta$  chain and the heavy chain or light chain of an immunoglobulin which do not form the heterodimer complex. However, since the molecules other than the heterodimer complex cannot be bound to a ligand, the supernatant solution can be used as a reagent for testing the binding to a ligand or cell, or searching for a substance inhibiting the binding between an integrin and a ligand, or for searching for a substance capable of being bound to an

integrin, or for measuring the ligand amount of an integrin. These methods of utilization are basically the same as those for using a purified integrin-immunoglobulin chimeric protein heterodimer complex described later.

An integrin-immunoglobulin chimeric protein heterodimer complex can be purified by an established method using a protein A column chromatography by use of the nature of the immunoglobulin portion. Furthermore, affinity chromatography using an antibody against the  $\alpha$  or  $\beta$  chain can also be used. Moreover, the purification can also be achieved by affinity chromatography with a ligand bound to a carrier. General chromatographic methods can also be used in combination for the purification. If publicly known cases in which integrin molecules are purified by these methods (Pytela, R. et al., Methods Enzymol., 144, 475-489 (1987), Santoro, S. A. et al., Biochem. Biophys. Res. Comm., 153, 217-223 (1988), Charo, I.F. et al., J. Cell Biol., 111, 2795-2800 (1990), Makarem, R. et al., J. Biol. Chem., 269, 4005-4011 (1994), Pfaff, M. et al., Eur. J. Immunol., 225, 975-984 (1994), Gulino, D. et al., Eur. J. Biochem., 227, 108-115 (1995), etc.) are applied, the purification of an integrin-immunoglobulin chimeric protein heterodimer complex can be achieved.

A purified integrin-immunoglobulin chimeric protein heterodimer complex can be identified as a protein showing at least one band under non-reducing condition and at least two

bands under reducing condition by SDS-PAGE. It can also be confirmed from it, that the heterodimer is linked by the disulfide bond between immunoglobulin heavy chains. It sometimes occurs that a plurality of bands are detected under reduction, but this is considered to be probably because intramolecular cleavage of the  $\alpha$  chain has occurred. Especially, with  $\alpha 4$ , this phenomenon is known (Hemler, M.E. et al., J. Biol. Chem., 262, 11478-11485 (1987)). Furthermore, it can be confirmed by the Western blotting method that the respective bands indicate chimeric proteins. As another method, it can be confirmed by said ELISA method combining an anti- $\alpha$  chain antibody, anti- $\beta$  chain antibody and anti-human immunoglobulin antibody, that the obtained molecule is an integrin-immunoglobulin chimeric protein heterodimer complex. That is, the molecule can be identified as a protein molecule with epitopes for all the antibodies. As a further other method, an integrin-immunoglobulin chimeric protein heterodimer complex can also be identified by immunoprecipitation. In this case, if the purified protein is labeled by  $^{35}\text{S}$ , or  $^{125}\text{I}$  or biotin, etc. according to any known method, and immunoprecipitated using an anti- $\alpha$  chain antibody, anti- $\beta$  chain antibody and anti-human immunoglobulin antibody, the same electrophoretic pattern can be obtained in every case. So, it can be confirmed that the integrin-immunoglobulin chimeric protein heterodimer complex has the

intended structure. Furthermore, even if a condition to dissociate the integrin complex on a cell membrane such as the coexistence of EDTA or boiling in the presence of SDS is applied, the immunoprecipitation pattern is not changed. So, it can be confirmed that the obtained integrin-immunoglobulin chimeric protein heterodimer complex is a structurally stabilized complex. The methods for confirming an integrin-immunoglobulin chimeric protein heterodimer complex are not limited to those stated above.

The binding between a prepared integrin-immunoglobulin chimeric protein heterodimer complex and a ligand can be tested as described below. After a ligand and an integrin-immunoglobulin chimeric protein heterodimer complex are brought into contact with each other, to make a mixture, the amount of the integrin-immunoglobulin chimeric protein heterodimer complex bound to the ligand or the amount of the ligand bound to the integrin-immunoglobulin chimeric protein heterodimer complex is measured. The amount of an integrin-immunoglobulin chimeric protein heterodimer complex can be measured by labeling the complex itself by a fluorescent dye or enzyme or radioisotope, etc. The amount of a ligand can also be measured by any similar method. A detection method such as SPA (Amasham) can also be used for the measurement. Furthermore, any reagent which can recognize a complex or ligand labeled by a fluorescent dye, enzyme or radioisotope,

etc. can also be used for the measurement. The reagent for recognizing an integrin-immunoglobulin chimeric protein heterodimer complex can, for example, be an anti-human immunoglobulin antibody. In this test, it is preferable to bind the molecule to be detected, to any carrier such as a bead or plate. As a ligand, its entire molecule can be used, but a portion retaining the binding activity to an integrin can also be taken out for use. For example, for integrin  $\alpha 4 \beta 1$  or integrin  $\alpha 2 \beta 1$ , its ligand, fibronectin or collagen or its peptide fragment bound to a carrier can also be used.

Methods similar to the above can be used to test the binding between an integrin-immunoglobulin chimeric protein heterodimer complex and cells. The amount of the cells bound to a complex can be measured by labeling the cells by a fluorescent dye or radioisotope or using a reagent reacting with the cells, for example, an antibody reacting with a surface antigen. If something like a tissue section is used instead of cells, the amount of the bound integrin-immunoglobulin chimeric protein heterodimer complex is measured by any of the above mentioned methods.

The methods for examining the binding between an integrin-immunoglobulin chimeric protein heterodimer complex and a ligand or cell described above can be used for obtaining a substance inhibiting the binding between an integrin and a ligand, for example, for obtaining an antibody, polypeptide,

peptide or low molecular weight compound. It is preferable to premix a sample and an integrin-immunoglobulin chimeric protein heterodimer complex, and then to measure the amount of the integrin-immunoglobulin chimeric protein heterodimer complex bound to a ligand in any of the above mentioned measuring systems. If the amount of the bound integrin-immunoglobulin chimeric protein heterodimer complex is lowered by adding a certain sample, it can be judged that the sample has inhibitory activity. However, in this system, a substance with metal ion chelating action or a substance with surfactant action, etc. may give a false positive result. The sources of samples used include the following integrin bound substances, peptide fragments of ligands, their derivatives, marketed compounds, etc., but are not limited to them.

A case where a purified integrin was coated on a plate to search for a peptide to be bound was reported (Healy, J. M. et al., Biochemistry 34, 3948-3955 (1995)). Even if the integrin-immunoglobulin chimeric protein heterodimer complex obtained in the present invention is used, a substance to be bound to an integrin can be similarly searched for. Especially when the chimeric protein heterodimer complex of the present invention is used, the operation to remove the non-specifically bound substances can be effected under more severe conditions. So, the operation can be simplified advantageously. Furthermore, since the complex is not

Patented 4/25/96

dissociated during operation, a bound substance can be selected more specifically advantageously. Known sources suitable for selecting bound substances include a phage peptide library (e.g., Scott, J. K. and Smith, G. P., Science, 249, 386-390 (1990)) and a DNA oligomer library (e.g., O'Connel, D. et al., Proc. Natl. Acad. Sci. USA, 93, 5883-5887 (1996), but in the present invention, it is preferable to use the former.

Furthermore, the method of testing the binding between an integrin-immunoglobulin chimeric protein heterodimer complex and a ligand or cell can also be used as a method for measuring the amount of an integrin ligand in a body fluid or tissue.

Moreover, the integrin-immunoglobulin chimeric protein heterodimer complexes of the present invention can also be used as drugs. The present invention has clarified that integrins and other isolated extracellular matrix receptors can be used as platelet substitutes.

An extracellular matrix receptor preferably used as a platelet substitute is an integrin. The  $\alpha$  chain of the integrin can be  $\alpha 1$ ,  $\alpha 2$ ,  $\alpha 3$ ,  $\alpha 4$ ,  $\alpha 5$ ,  $\alpha 6$ ,  $\alpha 7$ ,  $\alpha 8$ ,  $\alpha 9$ ,  $\alpha v$ ,  $\alpha L$ ,  $\alpha M$ ,  $\alpha X$ ,  $\alpha 11b$  or  $\alpha E$ , and among them,  $\alpha 2$  is preferable. The  $\beta$  chain can be  $\beta 1$ ,  $\beta 2$ ,  $\beta 3$ ,  $\beta 4$ ,  $\beta 5$ ,  $\beta 6$ ,  $\beta 7$  or  $\beta 8$ , and among them,  $\beta 1$  is preferable. Integrin  $\alpha 2 \beta 1$  is more preferable. The receptor source for isolation can be a tissue or cell expressing an

extracellular matrix receptor, or a dissolved membrane fraction of a receptor expressing cell prepared by gene manipulation, etc. It is more preferable to design for obtaining a soluble protein by modifying a receptor gene by gene recombination, and to use the cultured supernatant solution of the cells capable of producing it, as a source. Furthermore in the design of the soluble protein, it is preferable that the functional structure of the extracellular matrix receptor is retained. For example, it is desirable to use an integrin-immunoglobulin chimeric protein heterodimer complex obtained by modifying the heterodimer structure of an integrin to allow its  $\alpha$  and  $\beta$  chains to be covalently associated with each other. As the integrin-immunoglobulin chimeric protein heterodimer complex, it is preferable that the  $\alpha$  chain of the integrin is  $\alpha 1, \alpha 2, \alpha 3, \alpha 4, \alpha 5, \alpha 6, \alpha 7, \alpha 8, \alpha 9, \alpha v, \alpha L, \alpha M, \alpha X, \alpha 11b$  or  $\alpha E$ , and among them,  $\alpha 2$  is more preferable. Furthermore, it is preferable that the  $\beta$  chain is  $\beta 1, \beta 2, \beta 3, \beta 4, \beta 5, \beta 6, \beta 7$  or  $\beta 8$ , and among them,  $\beta 1$  is more preferable. It is further more preferable that the  $\alpha$  chain is  $\alpha 2$  and that the  $\beta$  chain is  $\beta 1$ . The platelet substitute of the present invention is described below mainly in reference to a typical extracellular matrix receptor, integrin  $\alpha 2 \beta 1$ -immunoglobulin chimeric protein heterodimer complex, but the present invention is not limited thereto or thereby.

To confirm the applicability of a purified integrin-

immunoglobulin chimeric protein heterodimer complex as a drug, the purified protein itself is used for examining its pharmacological activity. For obtaining higher capability of being bound to an extracellular matrix, it is more preferable to use an integrin-immunoglobulin chimeric protein heterodimer complex bound to a carrier such as a lipid or protein polymer, etc., but the present invention is not limited to this method.

For use as a platelet substitute, it is preferable to bind an integrin  $\alpha 2 \beta 1$ -immunoglobulin chimeric protein heterodimer complex to a liposome covalently according to the method stated in a report (Martin, F. J. et al., Biochemistry, 20, 4229 (1981)). The carrier can also be any other drug carrier than a liposome as far as its use for drugs is permitted. If a liposome is used as the carrier, the liposome is prepared according to the composition and method stated in a published book "Preparation and Experiments of Liposomes (in Japanese)", Oku, N. (1994), Hirokawa Shoten), but a preferable method is such that the epitope bound to the extracellular matrix of an integrin  $\alpha 2 \beta 1$ -immunoglobulin chimeric protein heterodimer complex is exposed outside the liposome membrane.

For confirming that an integrin  $\alpha 2 \beta 1$ -immunoglobulin chimeric protein heterodimer complex is bound on the prepared liposome carrier, a flow cytometer is used. The reagents which can be used for recognizing the integrin  $\alpha 2 \beta 1$ -immunoglobulin chimeric protein heterodimer complex include

an anti-integrin  $\alpha 2$  antibody, anti-integrin  $\beta 1$  antibody, anti-human immunoglobulin antibody, etc. If the antibody used is fluorescently labeled, it can be used for determination directly, but if it is not fluorescently labeled, a secondary antibody which recognizes the immunoglobulin class of the animal species used for preparing the antibody is used as a fluorescent label. As a further other confirmation method, the integrin  $\alpha 2 \beta 1$ -immunoglobulin chimeric protein heterodimer composite itself can be labeled by an enzyme or radioisotope, etc., for confirmation in proper combination with a color dye or radioactivity measuring instrument, etc.

To examine the extracellular matrix binding capability using an integrin  $\alpha 2 \beta 1$ -immunoglobulin chimeric protein heterodimer complex liposome, it is preferable to suspend the integrin  $\alpha 2 \beta 1$ -immunoglobulin chimeric protein heterodimer complex liposome into a buffer with a physiological cation concentration or plasma. The buffer with a physiological cation concentration refers to a buffer containing at least cations such as Mg ions or Ca ions and adjusted to about neutrality. The plasma is prepared by processing the blood collected in the presence of an anticoagulant, according to a general plasma preparation method. As the anticoagulant, for example, heparin or EDTA solution can be added by sufficient units. A marketed normal plasma, coagulation factor deficient plasma or serum, etc. can also be used. However, if the

anticoagulant used lowers the cation concentration, cations are added to achieve a physiological concentration later. Then, the integrin  $\alpha 2 \beta 1$ -immunoglobulin chimeric protein heterodimer complex liposome is mixed with an extracellular matrix or its fragment coated on a carrier for a certain time, to judge whether binding takes place. It is preferable that the coating of the extracellular matrix or its fragment as a solid phase is achieved by using a plastic plate, etc., but marketed beads for coating an extracellular matrix as a solid phase, etc. can also be used. When a collagen is used as the extracellular matrix, any animal species and type can be used. The binding reaction between an integrin  $\alpha 2 \beta 1$ -immunoglobulin chimeric protein heterodimer complex liposome and an extracellular matrix is effected according to a general method adopted for observing the adherence reaction of platelets. In many cases, they are allowed to stand mainly in a static system for a certain time, to induce binding to the matrix, but it is preferable to apply a shaking or shear stress, etc.

The integrin  $\alpha 2 \beta 1$ -immunoglobulin chimeric protein heterodimer complex liposome is bound to an extracellular matrix under the conditions as described above, and the amount of binding is measured by applying the above mentioned ELISA method using an anti-human immunoglobulin antibody. For more accurate determination, it is desirable to immobilize the liposome bound to the matrix by 1% glutaraldehyde, etc. As

another method than the ELISA method, for example, if a radio-labeled lipid is incorporated into the liposome beforehand, the amount of the liposome bound to the extracellular matrix can be obtained as radioactivity. Furthermore, to qualitatively judge the binding and covering degree to the extracellular matrix, a labeled antibody for recognizing the integrin  $\alpha 2 \beta 1$ -immunoglobulin chimeric protein heterodimer complex on the bound liposome can be combined with a color dye, etc., to dye the portions where the liposome is bound. It is more preferable that the generally used tissue antibody dyeing method is used to use a peroxidase labeled antibody against the integrin  $\alpha 2 \beta 1$ -immunoglobulin chimeric protein heterodimer complex and diaminobenzidine in combination, but the measuring method is not limited to it. As a further other method, the area covering the extracellular matrix can be obtained as a covering rate using an image processing analyzer.

Methods for examining the hemostasis of platelets include testing the adhering capability of platelets to the extracellular matrix and the agglutination capability induced by a collagen ("Handbook on the Examination of Blood Coagulation (in Japanese)", p. 65-78, Fukutake, M. and Fujimaki, M. (1987), Uchudo Yagi Shoten, Santro, S.A., Cell, 46, 913-920 (1986), Lethagen, S. and Rugarrn, P., Thrombo Haemost., 67, 185-186 (1982)). Especially the adhering

capability of platelets to the extracellular matrix is an indicator of primary hemostasis. The adhering capability is evaluated by using blood as it is, or platelet rich plasma or platelets washed by a buffer with physiological ions. Therefore, whether or not the integrin  $\alpha 2 \beta 1$ -immunoglobulin chimeric protein heterodimer complex liposome obtained in the present invention can be a functional substitute of platelets can be judged in reference its binding capability and the level of the binding capability to the extracellular matrix in the existence of plasma components or at a physiological ion concentration.

If the binding capability of the integrin  $\alpha 2 \beta 1$ -immunoglobulin chimeric protein heterodimer complex liposome obtained in the present invention to the extracellular matrix in the presence of the plasma components is strong, it suggests that the liposome can be a platelet substitute. Therefore, it can be used as a therapeutic or preventive agent against the congenital and acquired bleeding tendency due to platelet abnormality, and also widely as a platelet transfusion substitute.

Similarly the integrin  $\alpha 2 \beta 1$ -immunoglobulin chimeric protein heterodimer complex liposome obtained in the present invention can be a therapeutic or preventive agent for conditions of diseases where vascular endothelial cell disorder is a problem. For example, it was reported that in

the prognosis of PTCA (percutaneous coronary restenosis), the excessive accumulation of platelets on the extracellular matrix exposed by balloon catheter treatment triggers restenosis (Liu, M.W. et al., Circulation, 79, 1374-1378 (1989)). In Example 22, the effect of the integrin  $\alpha 2 \beta 1$ -immunoglobulin chimeric protein heterodimer complex liposome to cover the extracellular matrix was confirmed, and this effect can reduce the excessive accumulation of platelets to allow use also as a restenosis preventive. Furthermore, if the integrin  $\alpha 2 \beta 1$ -immunoglobulin chimeric protein heterodimer complex liposome is labeled by a medically allowable method, it can be used for monitoring the region of the extracellular matrix exposed by vascular endothelial cell injury, and furthermore, if a drug is enclosed in the liposome, it can also be applied to the targeting therapy for a local injured region.

When any integrin  $\alpha 2 \beta 1$ -immunoglobulin chimeric protein heterodimer complex liposome stated in the present invention is used as a platelet substitute, the administration paths include infusion, intravenous administration, etc., and it is usually used by being suspended in any physiologically suitable solution such as a salt solution or plasma, etc. It can be used alone or also in combination with another chimeric protein heterodimer complex with an extracellular matrix receptor or its immunoglobulin. It can also be used together

with another drug containing total platelets. The dose is properly selected to suit the symptom, age, body weight, etc., and can be 0.1 mg to 10 g per day as the amount of the protein for an adult, being able to be administered at a time or in several times. It can also be mixed with a pharmaceutically allowed carrier or excipient, etc., to be applied locally to the injured region as an externally applied drug such as an ointment, liniment or plaster. In this case, the externally applied drug is prepared to be 1 ng/cm<sup>2</sup> to 1 mg/cm<sup>2</sup> as the amount of the protein per one time of coating.

#### Examples

To describe the present invention in more detail, examples are given below. The general methods of recombinant DNA experiments conformed to those stated in a published book ("Antibody", Harlow, E. and Lane, D. (1988), Cold Spring Harbor Lab. Press, New York).

#### Example 1

##### Construction of human IgG<sub>1</sub> heavy chain expression vector

As human IgG<sub>1</sub> genome gene, a clone identical with reported base sequence information (Ellison, J. W. et al., Nucleic Acids Res., 10, 4071-4079 (1982)) was acquired from a human genomic library (CLONTECH) using a hybridization cDNA probe based on the sequence information. This was used as the template DNA for PCR. As primers for amplifying the DNA fragment containing the hinge region (H) and the constant

region portions (CH2 and CH3) of human IgG<sub>1</sub> gene, a DNA oligomer shown in sequence No. 4 of the sequence table (hereinafter a sequence No. of the sequence table is simply called a sequence No.) with BamH I restriction site and a DNA oligomer shown in sequence No. 5 with Xba I restriction site were synthesized.

5'-GCGGATCCCGAGCTGCTGGAAGCAGGCTCAG-3' (Sequence No. 4)

5'-CCTCTAGACGGCCGTCGCACTCATTTA-3' (Sequence No. 5)

The template DNA, primers, dNTPs (an equimolar mixture of dATP, dCTP, dGTP and dTTT) and Taq polymerase (Takara) were mixed in a PCR buffer (100 mM Tris-HCl, 500 mM KCl, 15 mM MgCl<sub>2</sub>, 0.01% gelatin, pH 8.3), and in a thermal cycler (Perkin Elmer Cetus), the mixture was treated at 94 °C for 1 minute for DNA denaturation, at 58°C for 2 minutes for annealing the primers and at 72°C for 3 minutes for elongating the primers. This treatment was performed 30 cycles. The amplified DNA was digested by restriction enzymes BamH I and Xba I, and the DNA fragment was purified by 1% agarose gel according to a general method ("Antibody", Harlow, E. and Lane, D. (1988), Cold Spring Harbor Lab. Press, New York). It was linked, using a T4DNA ligase, with a large DNA fragment of pBluescriptSK(+) (STRATAGENE) purified and digested by restriction enzymes BamH I and Xba I. The plasmid DNA was used to transform *Escherichia coli* (JM109), and the transformant was selected, to obtain a plasmid DNA (IgG<sub>1</sub> Bluescript). Then, expression

vector pcDL-SR  $\alpha$ 296 was digested by restriction enzyme BamH I, and blunted at the termini by T4DNA polymerase treatment, and a Not I linker was linked. The large DNA fragment obtained by digesting it by restriction enzymes Not I and Xho I and the small DNA fragment obtained by digesting IgG<sub>1</sub> Bluescript by restriction enzymes Not I and Xho I were purified according to a general method, and linked by T4DNA ligase. It was transformed into Escherichia coli (HB101), and the transformant was selected, to obtain a plasmid DNA. Hereinafter this plasmid (IgG<sub>1</sub>SR $\alpha$ ) is called human IgG<sub>1</sub> expression vector. In the following examples, since the basic protocol of gene manipulation is the same as above, the description will be simplified.

#### Example 2

##### Construction of integrin $\alpha$ 4-IgG heavy chain chimeric protein expression vector

The DNA fragment coding for the extracellular portion of integrin  $\alpha$ 4 was obtained by cloning based on reported cDNA sequence information (Takada, Y. et al., EMBO J., 8, 1361-1368 (1989)). The restriction site EcoR I of 1801-base-position of sequence No. 1, the restriction site Stu I of 112-base-position and the restriction site BamH I of 2949-base-position were used for linking the region from the N terminus translation initiation site to Stu I cut site as  $\alpha$ 4-1, the region from Stu I cut site to EcoR I cut site as  $\alpha$ 4-2, and the

region from EcoR I detached site to BamH I detached site as  $\alpha$  4-3. The detailed methods are described below.

The portion coding for  $\alpha$ 4-1 was designed to be cloned by linking the DNA oligomers of sequence Nos. 6 to 9, and the DNA oligomers shown in sequence Nos. 6 to 9 were synthesized. For the sequence Nos. 6 and 7, restriction site Xba I was added on the side to code for the N terminus, for linking to a vector. Furthermore, compared with the known sequence information, the bases at the 60-, 63- and 64-positions were substituted from C to T, C to A and C to G respectively, and the bases at the 112- and 114-positions were substituted from C to A and C to G respectively. Because of substitution at the 112- and 114-position, restriction site Stu I was inserted on the side to code for the N terminus of sequence Nos. 8 and 9. The 5' termini of the synthesized oligomers were phosphorylated and annealed, and were linked using T4DNA ligase. After completion of linking, restriction enzymes Xba I and Stu I were used for cutting, and electrophoresis was effected by 5% agarose (NuSieve GTGagarose, FMC) gel. The intended DNA fragment ( $\alpha$ 4-1) of about 120 bp was cut out and purified.

5' - CTAGACCACCATGTTCCCCACCGAGAGCGCATGGCTTGGGAAGCGAGGCGCAACCCGGGCCCCCGGA  
GCTGCA-3' (Sequence No. 6)

5' - GCTTCGGGGCCCCGGTTGCGCCTCGCTTCCCAAGCCATGCGCTCTCGGTGGGGAACATGGTGGT-3'  
(Sequence No. 7)

5' - CTCCGGGAGACGGTGATGCTGTTGCTGTGCCTGGGGGTCCCGACCGGCAGG-3'

(Sequence No. 8)

5' - CCTGCCGGTCGGGACCCCCAGGCACAGCAACAGCATCACCGTCTCCCGGAGTCGA-3 '

(Sequence No. 9)

Then, the RNA of human osteosarcoma cell line MG63 (ATCC CRL 1427) as an integrin  $\alpha 4$  expressing cell was separated, and PolyA(+)RNA was purified using oligo dT cellulose column (NEB). Based on it, a single stranded cDNA was synthesized using a reverse transcriptase (GIBCO), and used as the template for PCR. As primers for amplifying  $\alpha 4$ -2 and  $\alpha 4$ -3 DNAs, four DNA oligomers of sequence Nos. 10 to 13 with Pst I and Stu I restriction sites inserted (sequence No. 10) or BamH I restriction site inserted (sequence No. 13) were synthesized.

5' - CACTGCAGGCAGGCCTTACAACGTGGACACTGAGACC-3' (Sequence No. 10)

5' - GCAGAAACCTGTAAATCAGCAG-3' (Sequence No. 11)

5' - GCATTTATGCGGAAAGATGTGC-3' (Sequence No. 12)

5' - CGGGATCCGTGAAATAACGTTTGGGTCTT-3' (Sequence No. 13)

The template cDNA, primers, dNTPs and Taq polymerase were mixed in a PCR buffer, and the mixture was treated by a thermal cyclor at 94°C for 1 minute for DNA denaturation, at 58°C for 2 minutes for annealing the primers and at 72°C for 3 minutes for elongating the primers. This treatment was performed 30 cycles. The amplified DNA fragments of  $\alpha 4$ -2 and  $\alpha 4$ -3 were digested by Pst I and EcoR I respectively, or EcoR I

and BamH I and sub-cloned into pBluescriptKS(+) (STRATAGENE), to prepare plasmid DNAs (hereinafter called  $\alpha 4-2$  Bluescript and  $\alpha 4-3$  Bluescript). Then, upstream of the  $\alpha 4-2$  Bluescript,  $\alpha 4-1$  was linked using Xba I and Stu I restriction sites, to prepare a plasmid DNA (hereinafter called  $\alpha 4-1-2$  Bluescript).

The  $\alpha 4-1-2$  Bluescript was digested by restriction enzyme Not I, and blunted at the termini by T4DNA polymerase treatment, being digested by restriction enzyme EcoR I, to prepare a small DNA fragment. The  $\alpha 4-3$  Bluescript was digested by restriction enzymes EcoR I and BamH I, to prepare a small DNA fragment. The two small DNA fragments were simultaneously linked to a large DNA fragment obtained by digesting IgG<sub>1</sub>SR $\alpha$  by restriction enzymes EcoR V and BamH I, to obtain a plasmid DNA. The obtained base sequence coding for integrin  $\alpha 4$ ·IgG heavy chain chimeric protein is shown as sequence No. 1. The plasmid (integrin  $\alpha 4$ ·IgGSR $\alpha$ ) is hereinafter called integrin  $\alpha 4$ ·IgG heavy chain chimeric protein expression vector.

### Example 3

Construction of  $\beta 1$ ·IgG heavy chain chimeric protein expression vector

The RNA of human fibroblast cell line MRC5 (ATCC CCL 171) as an integrin  $\beta 1$  expressing cell was separated, and oligo dT cellulose column was used to purify PolyA (+)RNA. Based on it, a single stranded cDNA was synthesized using a reverse

transcriptase, and used as the template for PCR. As primers, two DNA oligomers of sequence Nos. 14 and 15 with BamH I site (sequence No. 15) inserted on the side coding for C terminus were synthesized according to the sequence information (Cott Argraves, W. et al., J. Cell Biol., 105, 1183-1190 (1987)).

5' - GCGGAAAAGATGAATTTACAAC-3' (Sequence No. 14)

5' - GTGGGATCCTCTGGACCAAGTGGGACAC-3' (Sequence No. 15)

The template cDNA, primers, dNTPs and Taq polymerase were mixed in a PCR buffer, and treated by a thermal cycler at 94°C for 1 minute for DNA denaturation, at 57°C for 2 minutes for annealing the primers and at 72°C for 3 minutes for elongating the primers. This treatment was performed 30 cycles. The amplified DNA was blunted at the termini by T4DNA polymerase treatment, and digested by restriction enzyme BamH I. Then, the DNA fragment was purified. Subsequently, the DNA fragment obtained in PCR before was sub-cloned at the Sma I and BamH I sites of pBluescriptKS(+). A small DNA fragment purified by digesting it by restriction enzymes EcoR I and BamH I was inserted into a large DNA fragment of IgG<sub>1</sub>SR $\alpha$  treated by restriction enzymes EcoR I and BamH I, to obtain a plasmid DNA. The obtained base sequence coding for  $\beta$ 1-IgG heavy chain chimeric protein is shown in sequence No. 2. The plasmid (integrin  $\beta$ 1-IgGSR $\alpha$ ) is hereinafter called integrin  $\beta$ 1-IgG heavy chain chimeric protein expression vector.

Example 4

Transfection of  $\alpha 4 \cdot \text{IgG}$  heavy chain chimeric protein expression vector and  $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein expression vector into animal cells, and their expression

Integrin  $\beta 1 \cdot \text{IgGSR}\alpha$  as  $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein expression vector and pSV2dhfr (BRL) were mixed at a ratio of 10 : 1, and the mixture and lipofectin reagent (GIBCO BRL) were gently mixed and allowed to stand at room temperature for 15 minutes. The mixture was added dropwise to dihydrofolic acid reductase deficient CHO cells (ATCC CRL 9096). After 18 hours of dropwise addition, the mixture was cultured in a medium (10%FBS (GIBCO), nucleic acid-containing  $\alpha$ MEM medium (GIBCO BRL)) for about 2 days, and the cells were dispersed by trypsin-EDTA treatment. The cells were suspended in a first selective medium (10% FBS-containing nucleic acid-free  $\alpha$ MEM medium (GIBCO BRL)), and the suspension was disseminated into a 96-well plate (CORNING), for selective culture for about 10 days. Then, the amount of integrin  $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein produced in the culture supernatant was determined according to the ELISA method (described later), and the clone showing the highest production was stabilized by cloning according to the limiting dilution method.

Then, into the stabilized integrin  $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein producing CHO cells, the integrin  $\alpha 4 \cdot \text{IgG}$  heavy chain chimeric protein expression vector was transfected

according to the lipofectin method as described before. That is, integrin  $\alpha 4$ ·IgGSR $\alpha$  and pSV2neo (BRL) were mixed at 10 : 1, and the mixture was mixed with lipofectin reagent. The mixture was added dropwise into the cells. After 18 hours of dropwise addition, the mixture was cultured in the said first selective medium for about 2 hours, and the cells were dispersed by trypsin-EDTA treatment. The cells were suspended in a second selective medium (nucleic acid-free  $\alpha$ MEM medium (GIBCO BRL) containing 10% FBS (GIBCO) and 1 mg/ml neomycin (GIBCO)), and on a 96-well plate (CORNING), resistant cells were selectively cultured for about 10 days. The amount of integrin  $\alpha 4$ ·IgG heavy chain chimeric protein and the amount of integrin  $\beta 1$ ·IgG heavy chain chimeric protein produced in the culture supernatant were determined according to the ELISA method (described later), and a clone which produced both the chimeric proteins by almost the same amounts was picked up. The clone was cloned twice according to the limiting dilution method, to be stabilized as a clone capable of producing  $\alpha 4$ ·IgG heavy chain- $\beta 1$ ·IgG heavy chain chimeric protein heterodimer complex.

#### Example 5

Determination of produced integrin  $\alpha 4$ ·IgG heavy chain chimeric protein and integrin  $\beta 1$ ·IgG heavy chain chimeric protein by the ELISA method

Fifty microliter per well of anti-human integrin  $\alpha 4$

antibody (Becton & Dickinson, Clone L25.3) or anti-human integrin  $\beta 1$  antibody (Coulter, Clone 4B4) ( $12 \mu\text{g/ml}$  each) was put into a 96-well immunoplate (NUNC), and allowed to stand at  $4^{\circ}\text{C}$  for 16 hours. Then, each well was washed by Dulbecco's phosphate buffered saline (Nissui Seiyaku, not containing Ca or Mg ions, hereinafter called PBS(-)) twice, and non-specific reaction was blocked by PBS(-) containing 25% Block Ace (Snow Brand Milk Products Co., Ltd.). After blocking, the culture supernatant of CHO cells grown in selective medium was properly diluted, and reacted with the coated antibody at room temperature for 1 hour. After the reaction, the surface of the plate was washed with 0.02% Tween-containing PBS(-) (hereinafter called T-PBS) twice. It was then caused to react with biotinized anti-human IgG antibody (Vector) for 1 hour, and the reaction mixture was washed with T-PBS twice, and in succession caused to react with avidin-horseradish peroxidase (Sigma) for 1 hour. The reaction mixture was washed with PBS(-) twice. The PBS(-) was perfectly aspirated, and orthophenylenediamine was used as a substrate for color development. The absorbance at 490 nm were measured using a microplate reader (Bio-rad NOVAPATH), and the clone showing a high absorbance value was selected.

#### Example 6

Purification of  $\alpha 4 \cdot \text{IgG}$  heavy chain- $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein heterodimer complex

(1) Culture of CHO cells and preparation of cultured supernatant solution

The CHO cells highly capable of producing the  $\alpha 4 \cdot \text{IgG}$  heavy chain- $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein heterodimer complex were cultured in nucleic acid-free  $\alpha \text{MEM}$  medium containing 5% FBS (Ultra-low IgG grade, GIBCO) (hereinafter called  $\alpha \text{MEM}(-)$  medium, GIBCO BRL) for one day, to reach semiconfluent, and they were cultured in  $\alpha \text{MEM}(-)$  medium containing 1% FBS (Ultra-low IgG grade) for 3 days, and the culture supernatant was collected. It was concentrated to 1/10 volume by ultrafiltration using Prep-scale (Millipore), and 1M Hepes solution (pH 8.0) was added to achieve a final concentration of 5 mM, for preparing a starting solution for further purification.

(2) Protein A column chromatography

The starting solution for further purification was passed through Prosep Guard column (bioPROCESSING), and applied to Prosep A column (bioPROCESSING). After completion of application, it was washed with 10 times the column volume of PBS(-), and the proteins were eluted at a pH 6 - 3 gradient of 0.1M citrate buffer solutions. The peak fraction eluted at pH 3 was collected, and 1M Tris-HCl solution (pH 8.5) was added by 0.1 volume for neutralization. The solution was dialyzed against PBS(-).

(3) Affinity column chromatography

FMP activated Cellulofine (Seikagaku Kogyo) was equilibrated by a coupling buffer (50 mM Na<sub>2</sub>CO<sub>3</sub>-NaHCO<sub>3</sub> pH 8.5), and a peptide showing sequence No. 3 (hereinafter called CS-1 peptide) synthesized by a peptide synthesizer was added. The mixture was inverted and mixed at 4 °C for 16 hours.

Cys Leu His Gly Pro Glu Ile Leu Asp Val Pro Ser Thr (Sequence No. 3)  
After completion of mixing, the mixture was washed with the coupling buffer, and a blocking buffer (0.1 mM monoethanolamine, 50 mM Tris-HCl, pH 8.0) was added. The mixture was inverted and mixed further at room temperature for 6 hours. Then, the mixture was sufficiently washed with TBS solution (150 mM NaCl, 20 mM Tris-HCl, 1 mM MnCl<sub>2</sub>, pH 7.5), to prepare CS-1 peptide bound Cellulofine column. To the column the starting solution for further purification was applied and allowed to stand at room temperature for 3 hours, and washed with 10 times the column volume of a washing buffer (1M NaCl, 0.1% Triton, 20 mM Tris-HCl, 1 mM MnCl<sub>2</sub>, pH 7.5) and the same volume of the TBS solution. After completion of washing, an elution buffer (10 mM EDTA, 150 mM NaCl, 20 mM Tris-HCl, pH 7.5) was used, to elute the proteins bound to the CS-1 column. The eluate was collected and dialyzed against PBS(-).

#### (4) SDS-PAGE

The eluted fractions of (3) were subjected to SDS-PAGE under non-reducing or reducing condition using 6.0 or 7.0% acrylamide gel, and the gel was stained with Coomassie-blue.

As a result, under non-reducing condition, two bands considered to be attributable to the  $\alpha 4 \cdot \text{IgG}$  heavy chain- $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein heterodimer complex and its polymer were observed. Under reducing condition, two bands (170 kDa and 135 kDa) considered to be attributable to the integrin  $\alpha 4 \cdot \text{IgG}$  heavy chain chimeric protein and the integrin  $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein and two bands (80 kDa and 90 kDa) considered to be attributable to the intramolecular cleavage of the integrin  $\alpha 4 \cdot \text{IgG}$  heavy chain chimeric protein (Hemler, M. E. et al., J. Biol. Chem., 262, 11478-11485 (1987)) were observed. These results suggest that the eluted protein of (3) has a molecular structure considered to be  $\alpha 4 \cdot \text{IgG}$  heavy chain- $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein heterodimer complex, and that the molecules constituting the heterodimer are linked by a disulfide bond between the IgG heavy chains.

#### Example 7

Identification of  $\alpha 4 \cdot \text{IgG}$  heavy chain- $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein heterodimer complex, and examination of its structural stability

(1) Immunoprecipitation using anti-integrin antibodies and influence of a cationic chelating agent

The basic method conformed to a published book ("Antibodies", Harlow, E. et al., (1988), Cold Spring Harbor Lab. Press, New York). That is, the eluted protein of Example

6 (3) considered to be  $\alpha 4 \cdot \text{IgG}$  heavy chain- $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein heterodimer complex was  $^{125}\text{I}$ -labeled using the lactoperoxidase method. Then, Affigel-10 (Bio-rad) was washed with 0.1 M Hepes solution (pH 8.0), and normal murine IgG, anti-human integrin  $\alpha 4$  antibody (clone 11C2B) and anti-human integrin  $\beta 1$  antibody (clone 4B4) were added. Reaction was effected at  $4^\circ\text{C}$  for 16 hours to cause covalent bonding, to prepare normal murine IgG beads and the respective antibody beads. Then, the  $^{125}\text{I}$  labeled  $\alpha 4 \cdot \text{IgG}$  heavy chain- $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein heterodimer complex and normal murine IgG beads were inverted and mixed at  $4^\circ\text{C}$  for 4 hours for preclearing, and the mixture and the antibody beads were inverted and mixed at  $4^\circ\text{C}$  for 16 hours. After completion of mixing, the beads were washed with a washing buffer (200 mM Tris-HCl, 0.5 M NaCl, 0.1% NP-40, 1 mM  $\text{MgCl}_2$  or 10 mM EDTA, pH 8.0) three times. After completion of washing, a sample buffer for electrophoresis was added to the beads for treatment at  $100^\circ\text{C}$  for 5 minutes, and the mixture was centrifuged. The supernatant solution was analysed by electrophoresis under reducing condition. After completion of electrophoresis, the gel was dried by a gel dryer, and the protein was detected by autoradiography.

As a result of immunoprecipitation in the presence of 1 mM  $\text{MgCl}_2$ , from the beads of both the anti-human integrin  $\alpha 4$  antibody and the anti-human integrin  $\beta 1$  antibody, the same

precipitation patterns expected from the structure of  $\alpha 4 \cdot \text{IgG}$  heavy chain- $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein heterodimer complex were obtained. Thus, the protein obtained in (3) of Example 6 was identified as  $\alpha 4 \cdot \text{IgG}$  heavy chain- $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein heterodimer complex.

On the other hand, the immunoprecipitation pattern obtained by using the anti-integrin  $\beta 1$  antibody beads in the presence of 10 mM EDTA was the same as that in the presence of 1 mM  $\text{MgCl}_2$ , to clarify that the association between integrin  $\alpha 4 \cdot \text{IgG}$  heavy chain chimeric protein and integrin  $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein does not depend on cations. The above results suggest that the eluted protein obtained in (3) of Example 6 was certain  $\alpha 4 \cdot \text{IgG}$  heavy chain- $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein heterodimer complex, and if the result of (4) of Example 6 is also taken into account, it is strongly suggested that the association between both the proteins is stable association through a disulfide bond existing the IgG heavy chains.

(2) Examination on the structural stability of  $\alpha 4 \cdot \text{IgG}$  heavy chain- $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein heterodimer complex by sequential immunoprecipitation

According to (1),  $^{125}\text{I}$  labeled  $\alpha 4 \cdot \text{IgG}$  heavy chain- $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein heterodimer complex was caused to react with normal murine IgG beads, anti-human integrin  $\alpha 4$  antibody (11C2B) beads or anti-human integrin  $\beta 1$  antibody

36477-455560

(4B4) beads at 4°C for 4 hours, and the reaction mixture was washed. After washing, the reaction mixture was boiled at 100°C for 5 minutes in the presence of 2% SDS and centrifuged, and the supernatant (primary immunoprecipitation sample) was diluted to 10 times by 1% BSA-containing PBS, and was again reacted with the anti-integrin  $\beta 1$  antibody beads and the anti-integrin  $\alpha 4$  antibody beads at 4°C for 16 hours. After completion of reaction, the beads were washed, and a sample buffer for electrophoresis was added. The mixture was treated at 100°C for 5 minutes and centrifuged. The supernatant solution (secondary immunoprecipitation sample) was analyzed by SDS-PAGE/autoradiography.

As a result, the electrophoretic pattern obtained by the primary immunoprecipitation was also similarly observed in the secondary immunoprecipitation. This result suggests that the association between the  $\alpha 4$ ·IgG heavy chain chimeric protein and the  $\beta 1$ ·IgG heavy chain chimeric protein in the  $\alpha 4$ ·IgG heavy chain- $\beta 1$ ·IgG heavy chain chimeric protein heterodimer complex is not dissociated either by boiling in the presence of 2% SDS, and strongly supports that the complex has a stable heterodimer structure based on a disulfide bond.

#### Example 8

Binding of  $\alpha 4$ ·IgG heavy chain- $\beta 1$ ·IgG heavy chain chimeric protein heterodimer complex to VCAM-1

It was examined that the  $\alpha 4$ ·IgG heavy chain- $\beta 1$ ·IgG

heavy chain chimeric protein heterodimer complex produced by CHO cells can be bound to the ligand of integrin  $\alpha 4 \beta 1$  by using the cells expressing VCAM-1. Human normal umbilical intravenous endothelial cells were cultured with IL-1 3U/ml for 16 hours, to prepare VCAM-1 expressing cells. The cells were treated by 1 mM EDTA at 37°C for 15 minutes, for dispersion as single cells. The cells ( $2 \times 10^5$  cells per sample tube) were cultured with the supernatant of the CHO cells producing  $\alpha 4$ ·IgG heavy chain- $\beta 1$ ·IgG heavy chain chimeric protein heterodimer complex for 30 minutes in the presence of 1 mM (final concentration)  $MnCl_2$  or 3 mM (final concentration) EDTA. After completion of reaction, the cells were washed twice by centrifugation at 1200 rpm at room temperature for 5 minutes using a buffer for binding assay (24 mM Tris-Cl, 10 mM Hepes, 150 mM NaCl, 1 mM  $MnCl_2$  or 1 mM EDTA, 1% BSA, 2 mM glucose, pH 7.4). After washing, FITC labeled-anti-human IgG antibody (Cappel) was added, and incubated at room temperature for 20 minutes. The cells were washed by the same buffer, and the chimeric proteins bound to the cells were determined by a flow cytometer (ELITE, Coulter).

The results are shown in Fig. 1. It was observed that the fluorescence intensity showing the binding of  $\alpha 4$ ·IgG heavy chain- $\beta 1$ ·IgG heavy chain chimeric protein heterodimer complex increased by culturing the VCAM-1 expression cells with the supernatant containing  $\alpha 4$ ·IgG heavy chain- $\beta 1$ ·IgG heavy chain

chimeric protein heterodimer complex. The binding was inhibited by adding anti-human integrin antibodies (anti- $\alpha$ -antibody: clone L25.3, 10  $\mu$ m/ml + anti- $\beta$ 1 antibody: clone 4B4, 10  $\mu$ m/ml) or 3 mM EDTA. This result suggests that the  $\alpha$ 4  $\cdot$  IgG heavy chain- $\beta$ 1  $\cdot$  IgG heavy chain chimeric protein heterodimer complex can be bound to VCAM-1 like the integrin  $\alpha$ 4 $\beta$ 1 existing on the surfaces of cell membranes, and furthermore that the binding is  $\alpha$ 4 $\beta$ 1-specific and retains a feature of the binding that it is dependent on cations.

#### Example 9

Binding of  $\alpha$ 4  $\cdot$  IgG heavy chain- $\beta$ 1  $\cdot$  IgG heavy chain chimeric protein heterodimer complex to a peptide fragment of fibronectin

The capability of  $\alpha$ 4  $\cdot$  IgG heavy chain- $\beta$ 1  $\cdot$  IgG heavy chain chimeric protein heterodimer complex to be bound to the peptide fragment (sequence No. 3) of the other ligand, fibronectin was also examined.

At first, according to the said report (Humphries, M. J. et al., J. Biol. Chem., 262, 6886-6892 (1987)), the peptide fragment of sequence No. 3 (CS-1 peptide) was bound to rabbit IgG (Sigma), to prepare CS-1-IgG. The CS-1-IgG was diluted by PBS(-), and put in a 96-well immunoplate (NUNC) by 100  $\mu$ l/well, and allowed to stand at 4°C for 16 hours, to be formed as a solid phase on the plate.

After completion of standing, the surface of the plate was

Biological Abstracts  
washed with PBS(-) twice and treated with denatured 1% BSA(heat-natured at 80°C for 10 minutes)-PBS solution ( 300  $\mu$ l/well) at 4°C for 3 hours to block the nonspecific reaction. Then, the solid phase CS-1-IgG and the CHO culture supernatant (100  $\mu$ l) containing  $\alpha$ 4-IgG heavy chain- $\beta$ 1-IgG heavy chain chimeric protein heterodimer complex were reacted with each other at 30°C for 3 hours. The non-bound  $\alpha$ 4-IgG heavy chain- $\beta$ 1-IgG heavy chain chimeric protein heterodimer complex was removed by washing with 0.1% BSA-containing TBS buffer (150 mM NaCl, 25 mM Tris-HCl, 1 mM MnCl<sub>2</sub>, pH 7.4) twice, and the bound  $\alpha$ 4-IgG heavy chain- $\beta$ 1-IgG heavy chain chimeric protein heterodimer complex was detected by biotin labeled anti-human IgG antibody (Vector) as the primary antibody and avidin labeled horseradish peroxidase (Sigma) as the secondary antibody. The surface of the plate was washed with the TBS buffer. Orthophenylenediamine was added as a substrate to it for color development, and the absorbance at 490 nm were measured.

The results are shown in Fig. 2. The reaction with  $\alpha$ 4-IgG heavy chain- $\beta$ 1-IgG heavy chain chimeric protein heterodimer complex showed a rise in the absorbance indicating the binding to CS-1 peptide. The binding was almost perfectly inhibited by the presence of anti-integrin  $\alpha$ A antibody (clone L25.3), anti-integrin  $\beta$ 1 antibody (clone 4B4) or 5 mM EDTA. Therefore, it was clarified that  $\alpha$ 4-IgG heavy chain- $\beta$ 1-IgG

heavy chain chimeric protein heterodimer complex can be bound also to the CS-1 peptide which is a peptide fragment of fibronectin, and that a feature of binding that it depends on cations is retained.

#### Example 10

Evaluation of an inhibitory peptide by using a system for determining the binding of  $\alpha 4 \cdot \text{IgG}$  heavy chain- $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein heterodimer complex to a peptide fragment of fibronectin

In the binding determination system of Example 9, the effects of three peptides, i.e., sequence No. 16 (hereinafter called GPEILDVPST), 17 (hereinafter called GPEILEVPST) and 18 (hereinafter called GRGDSP) were examined.

Gly Pro Glu Ile Leu Asp Val Pro Ser Thr	(Sequence No. 16)
Gly Pro Glu Ile Leu Glu Val Pro Ser Thr	(Sequence No. 17)
Gly Arg Gly Asp Ser Pro	(Sequence No. 18)

The all peptides were synthesized by a peptide synthesizer. The peptide and 100  $\mu\text{l}$  of CHO cultured supernatant solution containing  $\alpha 4 \cdot \text{IgG}$  heavy chain- $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein heterodimer complex were mixed at room temperature for 20 minutes, and the binding to CS-1-IgG was determined according to the method in Example 9. The results are shown in Fig. 3. GPEILDVPST showed temperature-dependent inhibitory activity in a range of 0.1 to 10  $\mu\text{mg/ml}$ , but GPEILEVPST and GRGDSP did not show any inhibition of the

binding. These results show that the binding determination system in Example 9 allows to detect the inhibiting effect of the peptide (GPEILDVPST) inhibiting the binding between integrin  $\alpha 4 \beta 1$  and CS-1 peptide specifically .

#### Example 11

Construction of integrin  $\alpha 2$  - IgG heavy chain chimeric protein expression vector

The DNA fragment coding for the extracellular portion of integrin  $\alpha 2$  was divided into  $\alpha 2$ -1 and  $\alpha 2$ -2 based on the reported cDNA sequence information (Takada, Y. et al., J. Cell. Biol., 109, 397-407 (1989)) and subcloned, and they were integrated on an expression vector. At first, the RNA of human fibroblast cell line MRC-5 (ATCC CCL 171) as integrin  $\alpha 2$  expressing cell was separated, and an oligo dT cellulose column was used to purify PolyA(+)RNA. Based on it, a single stranded cDNA was synthesized and used as the template of PCR. As PCR primers, DNA oligomers of sequence Nos. 20 and 21 were synthesized for  $\alpha 2$ -1, and DNA oligomers of sequence Nos. 22 and 23, for  $\alpha 2$ -2.

5' -GCTCGAGCAAACCCAGCGCAACTACGG-3' (Sequence No. 20)

5' -ATAGTGCCCTGATGACCATTG-3' (Sequence No. 21)

5' -GATGGCTTTAATGATGTGATTG-3' (Sequence No. 22)

5' -TGTTGGTACTTCGGCTTTCTC-3' (Sequence No. 23)

The template cDNA, primers, dNTPs and Taq polymerase were mixed in a PCR buffer and PCR was performed 30 cycles by a

thermal cycler (reaction conditions: 94°C 1 minute - 60°C 2 minutes - 72°C 3 minutes). The amplified DNA fragment of  $\alpha 2-1$  was digested by restriction enzymes Xho I and EcoR I, and the DNA fragment of  $\alpha 2-2$  was blunted at the termini by T4DNA polymerase treatment and digested by restriction enzyme EcoR I. Each fragment was purified. The two purified DNA fragments were caused to react in a phosphating reaction solution (50 mM Tris-HCl, 10 mM MgCl<sub>2</sub>, 25 mM DTT, 1 mM ATP, 0.1 U/ $\mu$ l T4 polynucleotide kinase (Takara), pH 8.0) at 37 °C for 1 hour, and the reaction mixture was heat-treated at 68°C for 5 minutes to inactivate the enzyme. Then, IgG<sub>1</sub>SR $\alpha$  prepared in Example 1 was digested by restriction enzyme BamH I and caused to react in Klenow reaction solution (66 mM Tris-HCl, 10 mM MgCl<sub>2</sub>, 10 mM DTT, 0.2 mM dNTPs, 0.05 U/  $\mu$ l Klenow fragment (Takara), pH 7.5) at 37°C for 30 minutes, to blunt the termini, and the reaction mixture was heat-treated at 70°C for 5 minutes to inactivate the enzyme. Furthermore, a large DNA fragment was digested by restriction enzyme Xho I, and purified. The two ( $\alpha 2-1$  and  $\alpha 2-2$ ) DNA fragments phosphated before were inserted into the large DNA fragment, to obtain a plasmid DNA. The obtained base sequence coding for integrin  $\alpha 2$ ·IgG heavy chain chimeric protein is shown in sequence No. 19. This plasmid (integrin  $\alpha 2$ ·IgGSR $\alpha$ ) is hereinafter called  $\alpha 2$ ·IgG heavy chain chimeric protein expression vector.

Example 12

Transfection of integrin  $\alpha 2 \cdot \text{IgG}$  heavy chain chimeric protein expression vector and integrin  $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein expression vector into animal cells, and their manifestation

The integrin  $\alpha 2 \cdot \text{IgG}$  heavy chain chimeric protein expression vector was transfected into the integrin  $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein producing CHO cells prepared and stabilized in Example 4, according to the lipofectin method described in Example 4. That is, integrin  $\alpha 2 \cdot \text{IgGSR}\alpha$  and pSV2neo (BRL) were mixed at 10 : 1, and the mixture was mixed with lipofectin reagent. The mixture was added dropwise to the cells. Eighteen hours after completion of dropwise addition, the mixture was cultured in a first selective medium for 2 days, and the cells were dispersed by trypsin-EDTA treatment. The cells were suspended in a second selective medium, and the suspension was disseminated into a 96-well plate. Resistant cells were selectively cultured for about 10 days. Then, the amount of integrin  $\alpha 2 \cdot \text{IgG}$  heavy chain chimeric protein and the amount of integrin  $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein produced in the culture supernatant were determined according to the ELISA method (described later), and a clone producing almost the same amounts of both the chimeric proteins was picked up. The clone was cloned twice according to the limiting dilution analysis, to be stabilized as a clone capable of producing  $\alpha$

2 · IgG heavy chain- $\beta$ 1 · IgG heavy chain chimeric protein heterodimer complex.

#### Example 13

Determination of the amounts of integrin  $\alpha$ 2 · IgG heavy chain chimeric protein and integrin  $\beta$ 1 · IgG heavy chain chimeric protein by the ELISA method

Fifty microliter per well of anti-human integrin  $\alpha$ 2 antibody (Becton & Dickinson, clone P1E6) or anti-human integrin  $\beta$ 1 antibody (clone 4B4) ( $2\mu\text{g/ml}$  each) was put into a 96-well immunoplate, and allowed to stand at  $4^{\circ}\text{C}$  for 16 hours. Then, each well was washed with PBS(-) twice, blocked, and the culture supernatant of the CHO cells grown in second selective medium was properly diluted and reacted with the coated-antibody at room temperature for 1 hour. After the reaction, the surface of the plate was washed with T-PBS twice, and caused to react with biotinated anti-human IgG antibody for 1 hour and with avidin-horseradish peroxidase for 1 hour, and the reaction mixture was washed with PBS(-) twice. After completion of reaction, orthophenylenediamine was used as a substrate for color development, and the absorbance values at 490 nm were measured using a microplate reader. A clone showing a high absorbance value was selected.

#### Example 14

Purification of  $\alpha$ 2 · IgG heavy chain- $\beta$ 1 · IgG heavy chain chimeric protein heterodimer complex

(1) Culture of CHO cells and preparation of cultured supernatant solution

The CHO cells highly capable of producing  $\alpha 2 \cdot \text{IgG}$  heavy chain- $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein heterodimer complex were cultured in an  $\alpha \text{MEM}(-)$  medium containing 5% FBS (Ultra-low IgG grade) for 1 day, to reach semiconfluent, and they were cultured on an  $\alpha \text{MEM}(-)$  medium containing 1% FBS (Ultra-low IgG grade) for 3 days. The culture supernatant was collected, and concentrated to 1/10 volume by ultrafiltration. Then, 1M Hepes solution (pH 8.0) was added to achieve a final concentration of 5 mM, to obtain a starting solution for further purification.

(2) Protein A column chromatography

The starting solution for further purification was passed through Prosep Guard column, and applied to Prosep A column. After completion of application, it was washed with 10 times the column volume of PBS (-), and in succession, the proteins were eluted at a pH 6 to 3 gradient of 0.1M citrate buffers. The peak fraction eluted at pH 3 was collected, and 1M Tris-HCl solution (pH 8.5) was added by 0.1 volume for neutralization. The mixture was dialyzed against PBS(-).

(3) Affinity column chromatography

According to a report (Kirchhofer, D. et al., J. Biol. Chem., 265, 615-618 (1990)), a collagen immobilized column with a collagen (Type I, Sigma) coupled to cyanogen-bromide-

activated Sepharose (Sigma) was prepared. Then, the starting solution for further purification was equilibrated in a TBS buffer (150 mM NaCl, 50 mM Tris-HCl, 1 mM MgCl<sub>2</sub>, 1 mM MnCl<sub>2</sub>, pH 7.5), applied to a column, allowed to stand at room temperature for 3 hours, and washed with 10 times the column volume of a washing buffer (150 mM NaCl, 50 mM Tris-HCl, 1 mM MgCl<sub>2</sub>, 1 mM MnCl<sub>2</sub>, 100 mM octyl glucopyranoside, pH 7.5). After completion of washing, an elution buffer (20 mM EDTA, 150 mM NaCl, 50 mM Tris-HCl, 50 mM octyl glucopyranoside, pH 7.5) was used to elute the protein bound to the column. The eluate was collected and dialyzed against PBS(-).

#### (4) SDS-PAGE

The eluted fraction of (3) was subjected to SDS-PAGE using 7.0% acrylamide gel under non-reducing or under reducing condition, and the gel was stained with Coomassie-blue. As a result, a band considered to be attributable to  $\alpha 2 \cdot \text{IgG}$  heavy chain- $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein heterodimer complex was observed. Under reducing condition, two bands (185 kDa and 135 kDa) considered to be attributable to integrin  $\alpha 2 \cdot \text{IgG}$  heavy chain chimeric protein and integrin  $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein were observed. These results suggest that the eluted protein has a molecular structure considered to be  $\alpha \alpha 2 \cdot \text{IgG}$  heavy chain- $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein heterodimer complex, and is linked by a disulfide bond between the IgG heavy chains.

#### Example 15

Identification of  $\alpha 2 \cdot \text{IgG}$  heavy chain- $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein heterodimer complex, and examination of its structural stability

The eluted protein of (3) of Example 14 was  $^{125}\text{I}$ -labeled, and subjected to immunoprecipitation using the beads coupled with normal murine IgG, anti-human integrin  $\alpha 2$  antibody (clone P1E6) or anti-human integrin  $\beta 1$  antibody (clone 4B4) as described in Example 7, and to SDS-PAGE/autoradiography under reducing condition.

As a result, in both 1 mM  $\text{MgCl}_2$  and 10 mM EDTA, from the beads of both anti-human integrin  $\alpha 2$  antibody and anti-human integrin  $\beta 1$  antibody, the same precipitation patterns expected from the structure of  $\alpha 2 \cdot \text{IgG}$  heavy chain- $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein heterodimer complex could be obtained. These results show that the eluted protein obtained in (3) of Example 14 is certainly  $\alpha 2 \cdot \text{IgG}$  heavy chain- $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein heterodimer complex, and with the results of (4) of Example 14 also taken into account, it is strongly suggested that the association of both the proteins is stable through a disulfide bond existing the IgG heavy chains.

#### Example 16

Examination on the capability of  $\alpha 2 \cdot \text{IgG}$  heavy chain- $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein heterodimer complex to be bound

to a collagen, and its specificity

The capability of  $\alpha 2 \cdot \text{IgG}$  heavy chain- $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein heterodimer complex to be bound to a collagen which is a ligand of integrin  $\alpha 2 \beta 1$  was examined.

At first, a collagen (Cell Matrix Type1 3 mg/ml) was diluted to 0.1  $\mu\text{g/ml}$  by 0.02M acetic acid solution, and put in an immunoplate by 100  $\mu\text{l/well}$ , being kept at 4°C for 16 hours. Then, the collagen solution was removed by suction, and the plate was washed with PBS(-) twice for neutralization. Heat-denaturated 1% BSA-PBS solution was put in the plate by 300  $\mu\text{l/well}$  for blocking at room temperature for 3 hours. After completion of blocking, it was rinsed with PBS(-) twice, to prepare a collagen coated plate.

The cultured supernatant of CHO (100  $\mu\text{l}$ ) containing  $\alpha 2 \cdot \text{IgG}$  heavy chain- $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein heterodimer complex was reacted at 30°C for 3 hours. After completion of reaction, as described in Example 9, the amount of bound  $\alpha 2 \cdot \text{IgG}$  heavy chain- $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein heterodimer complex was determined.

As a result, as shown in Fig. 4, the absorbance showing the binding of  $\alpha 2 \cdot \text{IgG}$  heavy chain- $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein heterodimer complex to the collagen was increased. The binding was almost perfectly inhibited in the coexistence of 10  $\mu\text{g/ml}$  of anti-human integrin  $\alpha 2$  antibody (clone P1E6) and anti-human integrin  $\beta 1$  antibody (clone 4B4),

or in the presence of 5 mM EDTA respectively. This result shows that  $\alpha 2 \cdot \text{IgG}$  heavy chain- $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein heterodimer complex can be bound to a collagen like the integrin  $\alpha 2 \beta 1$  existing on the surfaces of cell membranes, and furthermore that the binding is  $\alpha 2 \beta 1$ -specific and that the feature of the binding that it depends on cations is retained.

#### Example 17

Acquisition of a peptide capable of being bound to  $\alpha 4 \cdot \text{IgG}$  heavy chain- $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein heterodimer complex, and evaluation of its inhibitory activity

At first,  $\alpha 4 \cdot \text{IgG}$  heavy chain- $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein heterodimer complex purified in Example 6, or human IgG was prepared at a proper concentration by PBS(-) and was coated on a plastic plate at 4°C for 16 hours, being formed as a solid phase on a plastic plate. Then, according to a report (Cott, J. K. and Smoth, G. P., Science, 249, 386-390 (1990)), a phage peptide library in which a random six amino acid residues were cyclized by the disulfide bond of cysteine at both the ends was prepared and suspended in 0.1% BSA-containing TBS buffer. The phage peptide library was reacted with human IgG at 30°C for 3 hours, to absorb phage peptides capable of being bound to IgG. Then, the non-absorbed phases were reacted with  $\alpha 4 \cdot \text{IgG}$  heavy chain- $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein heterodimer complex at 30°C for 3 hours, and

the reaction mixture was washed with 0.1% BSA-containing TBS buffer twice to remove the phage peptides incapable of being bound to the heterodimer complex. Only the phage peptides capable of being bound were collected after elution with 0.1M glycine-hydrochloric acid (pH 2.2). After collection, the phage was amplified and the above mentioned binding operation was repeated further twice. The only the phage peptides capable of being bound to the heterodimer complex were selectively concentrated. In the final elution operation, phage peptides capable of being bound to the heterodimer complex were eluted using 10 mM EDTA and 0.1M glycine-hydrochloric acid in two steps, and the amino acid sequences of the respective peptides were analyzed. Of them, eight sequences (sequence Nos. 24 to 31) are shown in Table 1. Furthermore, they were examined using the binding assay system of Example 9, and the IC50 values of the four peptide sequences showing binding inhibitory activity are shown in Table 1.

Table 1

Elution condition	Sequence								Inhibitory activity IC50 ( $\mu$ M)	Sequence No.
EDTA	Cys*	Ile	Pro	Glu	Leu	Ile	Val	Cys*	1.2	24
	Cys*	Met	Arg	Tyr	Thr	Ser	Ala	Cys*	2.3	25
	Cys*	Glu	Trp	Met	Lys	Arg	Phe	Cys*		26
	Cys*	Tyr	Thr	Thr	Arg	Leu	Lys	Cys*		27

Glycine- hydrochloric acid	Cys*	Leu	Arg	Tyr	Ser	Val	Pro	Cys*	1.8	28
	Cys*	Ile	Val	Asn	Arg	Leu	Gly	Cys*		29
	Cys*	Gly	Leu	Gln	Ala	Leu	Pro	Cys*	10	30
	Cys*	Lys	Leu	Lys	Gly	Thr	Met	Cys*		31

Cys\* indicates a disulfide bond.

#### Example 18

Acquisition of a low weight molecular compound capable of inhibiting the binding between the peptide fragment on fibronectin and  $\alpha 4 \cdot \text{IgG}$  heavy chain- $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein heterodimer complex

Reagents and reported compounds were picked up at random, adjusted to a final concentration of 50 or 100  $\mu\text{g/ml}$ , and added to the binding determination system in Example 9. Compounds showing inhibitory activity were obtained. Of the obtained compounds, the binding inhibitory activities of the four compounds of Norethynodrel (Sigma), D-Penicillamine (Aldrich, Weigert, W. M. et al., Angew. Chem. Int. Ed. Eng., 14, 330-336 (1975),  $\gamma$ -2-Naphthyl butyric acid (Fieser, L. F. J. Am. Chem. Soc., 70, 3197-3203 (1948)), 1-Adamantaneacetic acid (Aldrich) were shown in Table 2.

Table 2

Name of compound	Concentration ( $\mu\text{g/ml}$ )	Inhibition rate (%)
Norethynodrel	50	28
D-Penicillamine	50	51
$\gamma$ -2-Naphtyl butyric acid	100	37
1-Adamantaneacetic acid	100	65

### Example 19

Preparation of  $\alpha 2 \cdot \text{IgG}$  heavy chain- $\beta 1 \cdot \text{IgG}$  heavy chain  
chimeric protein heterodimer complex liposome

A liposome was prepared according to the Martin et al.'s method (Martin, F. J. et al., Biochemistry, 20, 4229, (1981)). At first, an activated SH group was introduced into dipalmitoyl phosphatidyl ethanolamine (DPPE, Sigma) using di-crosslinking reagent N-succineimidyl 3-(2-pyridyldithio)propionate (SDPD, Sigma), to prepare pyridylthiopropionyl dipalmitoyl phosphatidyl ethanolamine (PDP-DPPE). The PDP-DPPE, dipalmitoyl phosphatidyl choline (DPPC) and cholesterol were mixed, to prepare a lipid film, and it was treated by a sonicator. Then, a filter was used to obtain a liposome uniform in diameter (PDP-DPPE liposome). Then,  $\alpha 2 \cdot \text{IgG}$  heavy chain- $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein heterodimer complex or human IgG (Cappel) used as a negative control were dissolved in a Hepes buffer (100 mM Hepes, 150 mM NaCl, pH 8.0), and SDPD was added for reaction for 30 minutes. The reaction solution was applied to PD-10 column (Pharmacia), and eluted by 0.1M acetic acid-sodium acetate buffer (pH 5.5). To the eluate, dithiothreitol was added for treatment for 20 minutes, and the mixture was applied to PD-10 column again and eluted by a Hepes buffer (100 mM Hepes, 150 mM NaCl, pH 8.0), to obtain SDPD coupled  $\alpha 2 \cdot \text{IgG}$  heavy chain- $\beta 1 \cdot \text{IgG}$  heavy chain

chimeric protein heterodimer complex. The SDPD modified heterodimer complex and the PDP-DPPE liposome were caused to react with each other at room temperature for 24 hours, and the reaction mixture was separated by Sepharose 4B column (Sigma). From the peak fraction,  $\alpha 2 \cdot \text{IgG}$  heavy chain- $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein heterodimer complex liposome was obtained.

The amount of  $\alpha 2 \cdot \text{IgG}$  heavy chain- $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein heterodimer complex bound on the liposome was determined by a densitometer (ATTO) after SDS-PAGE/Coomassie staining, and adjusted to final concentration of 1 mg/ml.

#### Example 20

Flow cytometry of  $\alpha 2 \cdot \text{IgG}$  heavy chain- $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein heterodimer complex liposome

$\alpha 2 \cdot \text{IgG}$  heavy chain- $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein heterodimer complex liposome was dispersed in 1 mM EDTA-containing PBS(-), and caused to react with anti-human integrin  $\alpha 2$  antibody (clone P1E6) or anti-human integrin  $\beta 1$  antibody (clone 4B4) at room temperature for 30 minutes. After completion of reaction, the reaction mixture was centrifuged at 15000 rpm for 10 minutes, being followed by washing with 1 mM EDTA-containing PBS(-) and suspended into the solution again. Into the suspension, FITC labeled anti-murine IgG antibody (Cappel, 10  $\mu\text{g/ml}$ ) was added as a secondary antibody, and reacted at room temperature for 30

minutes. After completion of reaction, the reaction mixture was similarly washed by centrifugation, and flow cytometry analysis(ELITE, Coulter) was performed.

As a result, the positive reactions for both the antibodies were detected, confirming that  $\alpha 2 \cdot \text{IgG}$  heavy chain- $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein heterodimer complex was bound on the liposome.

#### Example 21

Binding activity of  $\alpha 2 \cdot \text{IgG}$  heavy chain- $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein heterodimer complex to a collagen

A collagen (Cell Matrix Typel, 3 mg/ml) was diluted by 0.02M acetic acid solution, and the solution was put in an immunoplate by 100  $\mu\text{l}$ /well, being kept at 4°C for 16 hours. Then, the collagen solution was removed by suction, and the plate was washed with PBS(-) twice for neutralization, and heat-denaturated 1% BSA-PBS solution was put in the plate by 300  $\mu\text{l}$ /well for blocking at room temperature for 3 hours. After completion of blocking, the plate was rinsed with PBS(-) twice, to prepare a collagen coated plate.

Normal human plasma (George King) and von Willebrand's factor deficient (severe) plasma (George Kind) were treated with anti-human IgG antibody and protein A, and dialyzed against PBS(-) for 24 hours, to remove the contained sodium citrate. In order that the Ca ion and Mg ion concentration might be a physiological concentration in the blood when used,

CaCl<sub>2</sub> and MgCl<sub>2</sub> were added to achieve final concentrations of 1.2 mM and 0.2 mM respectively. Into the normal human plasma and von Willebrand's factor deficient plasma adjusted in cation concentration,  $\alpha 2$  · IgG heavy chain- $\beta 1$  · IgG heavy chain chimeric protein heterodimer complex liposome or human IgG liposome was suspended to achieve protein concentrations of 1 to 100 ng/ml. Any of the suspensions was put in the collagen coated plate by 100  $\mu$ l/well. The plate was shaken by a plate shaker at 100 rpm, for reaction at room temperature for 15 minutes. After completion of reaction, the non-bound liposome was removed by washing with a PB solution (1.2 mM CaCl<sub>2</sub>, 0.2 mM MgCl<sub>2</sub>, 1% BSA-containing PBS, pH 7.4), and the bound liposome was immobilized by 1% glutaraldehyde-PBS at room temperature for 30 minutes. After completion of immobilization, a heat-denaturated BSA-PBS solution was used for blocking at room temperature for 1 hour. Then, as described in Example 16, it was caused to react with biotin labeled human IgG antibody used as a primary antibody and avidin labeled horseradish peroxidase used as a secondary antibody, and washed with a TBS buffer. Into it, orthophenylenediamine was added as a substrate for color development, and the absorbance at 490 nm were measured. To examine the effect of 5 mM EDTA, anti-integrin  $\alpha 2$  antibody (clone P1E6, 10  $\mu$ g/ml) and anti-integrin  $\beta 1$  antibody (clone 4B4, 10  $\mu$ g/ml), it was caused to react with the liposome suspension at room temperature for 15 minutes

before reaction with the collagen.

The results are shown in Figs. 5 and 6. In the normal human plasma, the human IgG liposome as a negative control was not found to be bound to the collagen, but the binding of  $\alpha 2 \cdot$  IgG heavy chain- $\beta 1 \cdot$  IgG heavy chain chimeric protein heterodimer complex liposome to the collagen was increased with the concentration dependent on manner. Also when the von Willebrand's factor deficient plasma was used, equivalent binding was detected. Furthermore, the binding to the collagen observed when 30 ng/ml of  $\alpha 2 \cdot$  IgG heavy chain- $\beta 1 \cdot$  IgG heavy chain chimeric protein heterodimer complex liposome was added to the normal plasma was completely inhibited by adding EDTA as a cation chelating agent or the antibodies. The results show that in plasma with a physiological cation concentration,  $\alpha 2 \cdot$  IgG heavy chain- $\beta 1 \cdot$  IgG heavy chain chimeric protein heterodimer complex liposome is bound to a collagen like platelets, and strongly suggest that it can be a substitute of adhesive platelets, and can be a reagent for monitoring the collagen exposed region. Furthermore, it is indicated that since equivalent binding activity was shown also in von Willebrand's factor deficient plasma, the liposome can also be used in the plasma with coagulation abnormality such as von Willebrand's disease.

#### Example 22

Analysis of collagen covering state by  $\alpha 2 \cdot$  IgG heavy chain- $\beta$

1 · IgG heavy chain chimeric protein heterodimer complex  
liposome

Five microliters of a collagen solution was spotted at the center of each of the wells of a Lab-Tek chamber slide (Intermed, 8-well type, plastic) and allowed to stand for 16 hours, then washed and treated for blocking. Then, a suspension in which  $\alpha 2 \cdot$  IgG heavy chain- $\beta 1 \cdot$  IgG heavy chain chimeric protein heterodimer complex liposome was suspended in normal human plasma to achieve a protein concentration of 30 ng/ml as described in Example 21 was put in the slide by 200  $\mu$  l/well, for reaction under the same conditions. After completion of reaction, the non-bound liposome was removed by washing with a PB buffer, and the retained was immobilized and treated for blocking. Then, it was bound to biotin labeled anti-human IgG antibody as a primary antibody and with avidin labeled horseradish peroxidase as a secondary antibody, and was washed with a TBS buffer. After completion of washing, diaminobenzidine was added for staining, to observe the covering state of the  $\alpha 2 \cdot$  IgG heavy chain- $\beta 1 \cdot$  IgG heavy chain chimeric protein heterodimer complex liposome bound on the collagen.

With the human IgG liposome, the collagen coated portion was not stained, but with  $\alpha 2 \cdot$  IgG heavy chain- $\beta 1 \cdot$  IgG heavy chain chimeric protein heterodimer complex liposome, the collagen coated portion was entirely stained. Therefore,

since the  $\alpha 2 \cdot \text{IgG}$  heavy chain- $\beta 1 \cdot \text{IgG}$  heavy chain chimeric protein heterodimer complex liposome covered the collagen coated portion only, it was strongly suggested that the liposome could be a substitute of adhesive platelets.

#### Industrial Availability:

The present invention provides integrin-immunoglobulin chimeric protein heterodimer complexes in which the  $\alpha$  chain and the  $\beta$  chain of an integrin are stably associated. The obtained integrin-immunoglobulin chimeric protein heterodimer complexes can be directly used as drugs, and can also be used for determining the binding between an integrin and a ligand, and searching for a substance capable of being bound to an integrin and a substance inhibiting the binding between an integrin and a ligand. They can also be used as diagnostic reagents.

Furthermore, among the heterodimer complexes, especially integrin  $\alpha 2 \beta 1$ -immunoglobulin chimeric protein heterodimer complex can be used as a substitute of platelets. Furthermore, integrin  $\alpha 2 \beta 1$ -immunoglobulin chimeric protein heterodimer complex can be used as a therapeutic or preventive agent for bleeding tendency involved in thrombocytopenia, platelet function abnormality, etc. Furthermore, it can also be used as a reagent for monitoring the exposed region of an extracellular matrix and for the targeting therapy.

# Sequence Table

Sequence No. 1

Length of sequence: 4228

Type of sequence: Nucleic acid

Sequence

ATG TTC CCC ACC GAG AGC GCA TGG CTT GGG AAG CGA GGC GCG AAC CCG	48
Met Phe Pro Thr Glu Ser Ala Trp Leu Gly Lys Arg Gly Ala Asn Pro	
-35 -30 -25	
GGC CCC GAA GCT GCA CTC CGG GAG ACG GTG ATG CTG TTG CTG-TGC CTG	96
Gly Pro Glu Ala Ala Leu Arg Glu Thr Val Met Leu Leu Leu Cys Leu	
-20 -15 -10	
GGG GTC CCG ACC GGC AGG CCT TAC AAC GTG GAC ACT GAG AGC GCG CTG	144
Gly Val Pro Thr Gly Arg Pro Tyr Asn Val Asp Thr Glu Ser Ala Leu	
-5 1 5	
CTT TAC CAG GGC CCC CAC AAC ACG CTG TTC GGC TAC TCG GTC GTG CTG	192
Leu Tyr Gln Gly Pro His Asn Thr Leu Phe Gly Tyr Ser Val Val Leu	
10 15 20 25	
CAC AGC CAC GGG GCG AAC CGA TGG CTC CTA GTG GGT GCG CCC ACT GCC	240
His Ser His Gly Ala Asn Arg Trp Leu Leu Val Gly Ala Pro Thr Ala	
30 35 40	
AAC TGG CTC GCC AAC GCT TCA GTG ATC AAT CCC GGG GCG ATT TAC AGA	288
Asn Trp Leu Ala Asn Ala Ser Val Ile Asn Pro Gly Ala Ile Tyr Arg	
45 50 55	
TGC AGG ATC GGA AAG AAT CCC GGC CAG ACG TGC GAA CAG CTC CAG CTG	336
Cys Arg Ile Gly Lys Asn Pro Gly Gln Thr Cys Glu Gln Leu Gln Leu	
60 65 70	
GGT AGC CCT AAT GGA GAA CCT TGT GGA AAG ACT TGT TTG GAA GAG AGA	384
Gly Ser Pro Asn Gly Glu Pro Cys Gly Lys Thr Cys Leu Glu Glu Arg	
75 80 85	

CAC AAT CAG TGG TTG GGG GTC ACA CTT TCC AGA CAG CCA GGA GAA AAT	432
Asp Asn Gln Trp Leu Gly Val Thr Leu Ser Arg Gln Pro Gly Glu Asn	
90 95 100 105	
GGA TCC ATC GTG ACT TGT GGG CAT AGA TGG AAA AAT ATA TTT TAC ATA	480
Gly Ser Ile Val Thr Cys Gly His Arg Trp Lys Asn Ile Phe Tyr Ile	
110 115 120	
AAG AAT GAA AAT AAG CTC CCC ACT GGT GGT TGC TAT GGA GTG CCC CCT	528
Lys Asn Glu Asn Lys Leu Pro Thr Gly Gly Cys Tyr Gly Val Pro Pro	
125 130 135	
GAT TTA CGA ACA GAA CTG AGT AAA AGA ATA GCT CCG TGT TAT CAA GAT	576
Asp Leu Arg Thr Glu Leu Ser Lys Arg Ile Ala Pro Cys Tyr Gln Asp	
140 145 150	
TAT GTG AAA AAA TTT GGA GAA AAT TTT GCA TCA TGT CAA GCT GGA ATA	624
Tyr Val Lys Lys Phe Gly Glu Asn Phe Ala Ser Cys Gln Ala Gly Ile	
155 160 165	
TCC AGT TTT TAC ACA AAG GAT TTA ATT GTG ATG GGG GCC CCA GGA TCA	672
Ser Ser Phe Tyr Thr Lys Asp Leu Ile Val Met Gly Ala Pro Gly Ser	
170 175 180 185	
TCT TAC TGG ACT GGC TCT CTT TTT GTC TAC AAT ATA ACT ACA AAT AAA	720
Ser Tyr Trp Thr Gly Ser Leu Phe Val Tyr Asn Ile Thr Thr Asn Lys	
190 195 200	
TAC AAG GCT TTT TTA GAC AAA CAA AAT CAA GTA AAA TTT GGA AGT TAT	768
Tyr Lys Ala Phe Leu Asp Lys Gln Asn Gln Val Lys Phe Gly Ser Tyr	
205 210 215	
TTA GGA TAT TCA GTC GGA GCT GGT CAT TTT CGG AGC CAG CAT ACT ACC	816
Leu Gly Tyr Ser Val Gly Ala Gly His Phe Arg Ser Gln His Thr Thr	
220 225 230	
GAA GTA GTC GGA GGA GCT CCT CAA CAT GAG CAG ATT GGT AAG GCA TAT	864
Glu Val Val Gly Gly Ala Pro Gln His Glu Gln Ile Gly Lys Ala Tyr	
235 240 245	

ATA TTC AGC ATT GAT GAA AAA GAA CTA AAT ATC TTA CAT GAA ATG AAA	912
Ile Phe Ser Ile Asp Glu Lys Glu Leu Asn Ile Leu His Glu Met Lys	
250 255 260 265	
GGT AAA AAG CTT GGA TCG TAC TTT GGA GCT TCT GTC TGT GCT GTG GAC	960
Gly Lys Lys Leu Gly Ser Tyr Phe Gly Ala Ser Val Cys Ala Val Asp	
270 275 280	
CTC AAT GCA GAT GGC TTC TCA GAT CTG CTC GTG GGA GCA CCC ATG CAG	1008
Leu Asn Ala Asp Gly Phe Ser Asp Leu Leu Val Gly Ala Pro Met Gln	
285 290 295	
AGC ACC ATC AGA GAG GAA GGA AGA GTG TTT GTG TAC ATC AAC TCT GGC	1056
Ser Thr Ile Arg Glu Glu Gly Arg Val Phe Val Tyr Ile Asn Ser Gly	
300 305 310	
TCG GGA GCA GTA ATG AAT GCA ATG GAA ACA AAC CTC GTT GGA AGT GAC	1104
Ser Gly Ala Val Met Asn Ala Met Glu Thr Asn Leu Val Gly Ser Asp	
315 320 325	
AAA TAT GCT GCA AGA TTT GGG GAA TCT ATA GTT AAT CTT GGC GAC ATT	1152
Lys Tyr Ala Ala Arg Phe Gly Glu Ser Ile Val Asn Leu Gly Asp Ile	
330 335 340 345	
GAC AAT GAT GGC TTT GAA GAT GTT GCT ATC GGA GCT CCA CAA GAA GAT	1200
Asp Asn Asp Gly Phe Glu Asp Val Ala Ile Gly Ala Pro Gln Glu Asp	
350 355 360	
GAC TTG CAA GGT GCT ATT TAT ATT TAC AAT GGC CGT GCA GAT GGG ATC	1248
Asp Leu Gln Gly Ala Ile Tyr Ile Tyr Asn Gly Arg Ala Asp Gly Ile	
365 370 375	
TCG TCA ACC TTC TCA CAG AGA ATT CAA GGA CTT CAG ATC AGC AAA TCG	1296
Ser Ser Thr Phe Ser Gln Arg Ile Glu Gly Leu Gln Ile Ser Lys Ser	
380 385 390	
TTA AGT ATG TTT GGA CAG TCT ATA TCA CGA CAA ATT GAT GCA GAT AAT	1344
Leu Ser Met Phe Gly Gln Ser Ile Ser Gly Gln Ile Asp Ala Asp Asn	
395 400 405	



CTT CAG CAG AAG AAA GAA AAA GAC ATA ATG AAA AAA ACA ATA AAC TTT	1872
Leu Gln Gln Lys Lys Glu Lys Asp Ile Met Lys Lys Thr Ile Asn Phe	
570 575 580 585	
GCA AGG TTT TGT GCC CAT GAA AAT TGT TCT GCT GAT TTA CAG GTT TCT	1920
Ala Arg Phe Cys Ala His Glu Asn Cys Ser Ala Asp Leu Gln Val Ser	
590 595 600	
GCA AAG ATT GGG TTT TTG AAG CCC CAT GAA AAT AAA ACA TAT CTT GCT	1968
Ala Lys Ile Gly Phe Leu Lys Pro His Glu Asn Lys Thr Tyr Leu Ala	
605 610 615	
GTT GGG AGT ATG AAG ACA TTG ATG TTG AAT GTG TCC TTG TTT AAT GCT	2016
Val Gly Ser Met Lys Thr Leu Met Leu Asn Val Ser Leu Phe Asn Ala	
620 625 630	
GCA GAT GAT GCA TAT GAA ACG ACT CTA CAT GTC AAA CTA CCC GTG GCT	2064
Gly Asp Asp Ala Tyr Glu Thr Thr Leu His Val Lys Leu Pro Val Gly	
635 640 645	
CTT TAT TTC ATT AAG ATT TTA GAG CTG GAA GAG AAG CAA ATA AAC TGT	2112
Leu Tyr Phe Ile Lys Ile Leu Glu Leu Glu Glu Lys Gln Ile Asn Cys	
650 655 660 665	
GAA GTC ACA GAT AAC TCT GGC GTG GTA CAA CTT GAC TGC AGT ATT GGC	2160
Glu Val Thr Asp Asn Ser Gly Val Val Gln Leu Asp Cys Ser Ile Gly	
670 675 680	
TAT ATA TAT GTA GAT CAT CTC TCA AGG ATA GAT ATT AGC TTT CTC CTG	2208
Tyr Ile Tyr Val Asp His Leu Ser Arg Ile Asp Ile Ser Phe Leu Leu	
685 690 695	
GAT GTG AGC TCA CTC AGC AGA GCG GAA GAG GAC CTC AGT ATC ACA GTG	2256
Asp Val Ser Ser Leu Ser Arg Ala Glu Glu Asp Leu Ser Ile Thr Val	
700 705 710	
CAT GCT ACC TGT GAA AAT GAA GAG GAA ATG GAC AAT CTA AAG CAC AGC	2304
His Ala Thr Cys Glu Asn Glu Glu Glu Met Asp Asn Leu Lys His Ser	
715 720 725	

AGA GTG ACT GTA GCA ATA CCT TTA AAA TAT GAG GTT AAG CTG ACT GTT	2352
Arg Val Thr Val Ala Ile Pro Leu Lys Tyr Glu Val Lys Leu Thr Val	
730                      735                      740                      745	
CAT GGG TTT GTA AAC CCA ACT TCA TTT GTG TAT GGA TCA AAT GAT GAA	2400
His Gly Phe Val Asn Pro Thr Ser Phe Val Tyr Gly Ser Asn Asp Glu	
750                      755                      760	
AAT GAG CCT GAA ACG TGC ATG GTG GAG AAA ATG AAC TTA ACT TTC CAT	2448
Asn Glu Pro Glu Thr Cys Met Val Glu Lys Met Asn Leu Thr Phe His	
765                      770                      775	
GTT ATC AAC ACT GGC AAT AGT ATG GCT CCC AAT GTT AGT GTG GAA ATA	2496
Val Ile Asn Thr Gly Asn Ser Met Ala Pro Asn Val Ser Val Glu Ile	
780                      785                      790	
ATG GTA CCA AAT TCT TTT AGC CCC CAA ACT GAT AAG CTG TTC AAC ATT	2588
Met Val Pro Asn Ser Phe Ser Pro Gln Thr Asp Lys Leu Phe Asn Ile	
795                      800                      805	
TTG GAT GTC CAG ACT ACT ACT GGA GAA TGC CAC TTT GAA AAT TAT CAA	2592
Leu Asp Val Gln Thr Thr Thr Gly Glu Cys His Phe Glu Asn Tyr Gln	
810                      815                      820                      825	
AGA GTG TGT GCA TTA GAG CAG CAA AAG AGT GCA ATG CAG ACC TTG AAA	2640
Arg Val Cys Ala Leu Glu Gln Gln Lys Ser Ala Met Gln Thr Leu Lys	
830                      835                      840	
GGC ATA GTC CGG TTC TTG TCC AAG ACT GAT AAG AGG CTA TTG TAC TGC	2688
Gly Ile Val Arg Phe Leu Ser Lys Thr Asp Lys Arg Leu Leu Tyr Cys	
845                      850                      855	
ATA AAA GCT GAT CCA CAT TGT TTA AAT TTC TTG TGT AAT TTT GGG AAA	2736
Ile Lys Ala Asp Pro His Cys Leu Asn Phe Leu Cys Asn Phe Gly Lys	
860                      865                      870	
ATG GAA AGT GGA AAA GAA GCC AGT GTT CAT ATC CAA CTG GAA GGC CGG	2784
Met Glu Ser Gly Lys Glu Ala Ser Val His Ile Gln Leu Glu Gly Arg	
875                      880                      885	

CCA TCC ATT TTA GAA ATG GAT GAG ACT TCA GCA CTC AAG TTT GAA ATA	2832
Pro Ser Ile Leu Glu Met Asp Glu Thr Ser Ala Leu Lys Phe Glu Ile	
890 895 900 905	
AGA GCA ACA GGT TTT CCA GAG CCA AAT CCA AGA GTA ATT GAA CTA AAC	2880
Arg Ala Thr Gly Phe Pro Glu Pro Asn Pro Arg Val Ile Glu Leu Asn	
910 915 920	
AAG GAT GAG AAT GTT GCG CAT GTT CTA CTG GAA GGA CTA CAT CAT CAA	2928
Lys Asp Glu Asn Val Ala His Val Leu Leu Glu Gly Leu His His Gln	
925 930 935	
AGA CCC AAA CGT TAT TTC ACG GAT CCC GAG CTGCTGGAAG CAGGCTCAGC	2978
Arg Pro Lys Arg Tyr Phe Thr Asp Pro Glu	
940 945	
GCTCCTGCCT GGACGCATCC CGGCTATGCA GCCCCAGTCC AGGGCAGCAA GGCAGGCCCC	3038
GTCTGCCTCT TCACCCGGAG CCTCTGCCCC CCCCACATCAT GCTCAGGGAG AGGGTCTTCT	3098
GGCTTTTTTCC CAGGCTCTGG GCAGGCACAG GCTAGGTGCC CCTAACCCAG GCCCTGCACA	3158
CAAAGGGGCA GGTGCTGGGC TCAGACCTGC CAAGAGCCAT ATCCGGGAGG ACCCTGCCCC	3218
TGACCTAAGC CCACCCCAA GGCCAAACTC TCCACTCCCT CAGCTCGGAC ACCTTCTCTC	3278
CTCCCAGATT CCAGTAACTC CCAATCTTCT CTCTGCA GAG CCC AAA TCT TGT GAC	3333
Glu Pro Lys Ser Cys Asp	
950	
AAA ACT CAC ACA TGC CCA CCG TGC CCA GGTAAGCCAG CCCAGGCCTC	3380
Lys Thr His Thr Cys Pro Pro Cys Pro	
955 960	
GCCCTCCAGC TCAAGGCGGG ACAGGTGCCC TAGAGTAGCC TGCATCCAGG GACAGGCCCC	3440
AGCCGGGTGC TGACACGTCC ACCTCCATCT CTTCCTCA GCA CCT GAA CTC CTG	3493
Ala Pro Glu Leu Leu	
965	
GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC	3541
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu	
970 975 980	

ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC 3589  
 Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser  
 985 990 995  
 CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG 3637  
 His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu  
 1000 1005 1010 1015  
 GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG 3685  
 Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr  
 1020 1025 1030  
 TAC CGG GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT 3733  
 Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn  
 1035 1040 1045  
 GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA GCC CCC 3781  
 Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro  
 1050 1055 1060  
 ATC GAG AAA ACC ATC TCC AAA GCC AAA GGTGGGACCC GTGGGGTGCG 3828  
 Ile Glu Lys Thr Ile Ser Lys Ala Lys  
 1065 1070  
 AGGGCCACAT GGACAGAGGC CGGCTCGGCC CACCCTCTGC CCTGAGAGTG ACCGCTGTAC 3888  
 CAACCTCTGT CCTACA GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG 3937  
 Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu  
 1075 1080  
 CCC CCA TCC CGG GAT GAG CTG ACC AAG AAC CAG GTC AGC CTG ACC TGC 3985  
 Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys  
 1085 1090 1095  
 CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC 4033  
 Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser  
 1100 1105 1110 1115

AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAT	4081
Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp	
1120 1125 1130	
TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG GAC AAG AGC	4129
Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser	
1135 1140 1145	
AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT	4177
Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala	
1150 1155 1160	
CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG-GGT AAA	4225
Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys	
1165 1170 1175	
TGA	4228

Sequence No. 2

Length of sequence: 3463

Type of sequence: Nucleic acid

Sequence

ATG AAT TTA CAA CCA ATT TTC TGG ATT GGA CTG ATC AGT TCA GTT TGC	48
Met Asn Leu Gln Pro Ile Phe Trp Ile Gly Leu Ile Ser Ser Val Cys	
-20 -15 -10 -5	
TGT GTG TTT GCT CAA ACA GAT GAA AAT AGA TGT TTA AAA GCA AAT GCC	96
Cys Val Phe Ala Gln Thr Asp Glu Asn Arg Cys Leu Lys Ala Asn Ala	
1 5 10	
AAA TCA TGT GGA GAA TGT ATA CAA GCA GGG CCA AAT TGT GGG TGG TGC	144
Lys Ser Cys Gly Glu Cys Ile Gln Ala Gly Pro Asn Cys Gly Trp Cys	
15 20 25	
ACA AAT TCA ACA TTT TTA CAG GAA GGA ATG CCT ACT TCT GCA CGA TGT	192
Thr Asn Ser Thr Phe Leu Gln Glu Gly Met Pro Thr Ser Ala Arg Cys	
30 35 40	

GAT GAT TTA GAA GCC TTA AAA AAG AAG GGT TGC CCT CCA GAT GAC ATA	240
Asp Asp Leu Glu Ala Leu Lys Lys Lys Gly Cys Pro Pro Asp Asp Ile	
45 50 55 60	
GAA AAT CCC AGA GGC TCC AAA GAT ATA AAG AAA AAT AAA AAT GTA ACC	288
Glu Asn Pro Arg Gly Ser Lys Asp Ile Lys Lys Asn Lys Asn Val Thr	
65 70 75	
AAC CGT AGC AAA GGA ACA GCA GAG AAG CTC AAG CCA GAG GAT ATT CAT	336
Asn Arg Ser Lys Gly Thr Ala Glu Lys Leu Lys Pro Glu Asp Ile His	
80 85 90	
CAG ATC CAA CCA CAG CAG TTG GTT TTG CGA TTA AGA TCA GGG GAG CCA	384
Gln Ile Gln Pro Gln Gln Leu Val Leu Arg Leu Arg Ser Gly Glu Pro	
95 100 105	
CAG ACA TTT ACA TTA AAA TTC AAG AGA GCT GAA GAC TAT CCC ATT GAC	432
Gln Thr Phe Thr Leu Lys Phe Lys Arg Ala Glu Asp Tyr Pro Ile Asp	
110 115 120	
CTC TAC TAC CTT ATG GAC CTG TCT TAT TCA ATG AAA GAC GAT TTG GAG	480
Leu Tyr Tyr Leu Met Asp Leu Ser Tyr Ser Met Lys Asp Asp Leu Glu	
125 130 135 140	
AAT GTA AAA AGT CTT GGA ACA GAT CTG ATG AAT GAA ATG AGG AGG ATT	528
Asn Val Lys Ser Leu Gly Thr Asp Leu Met Asn Glu Met Arg Arg Ile	
145 150 155	
ACT TCG GAC TTC AGA ATT GGA TTT GGC TCA TTT GTG GAA AAG ACT GTG	576
Thr Ser Asp Phe Arg Ile Gly Phe Gly Ser Phe Val Glu Lys Thr Val	
160 165 170	
ATG CCT TAC ATT AGC ACA ACA CCA GCT AAG CTC AGG AAC CCT TGC ACA	624
Met Pro Tyr Ile Ser Thr Thr Pro Ala Lys Leu Arg Asn Pro Cys Thr	
175 180 185	
AGT GAA CAG AAC TGC ACC ACC CCA TTT AGC TAC AAA AAT GTG CTC AGT	672
Ser Glu Gln Asn Cys Thr Thr Pro Phe Ser Tyr Lys Asn Val Leu Ser	
190 195 200	



GAA AAC CGC AAA TTG TCA GAA GGA GTA ACA ATA AGT TAC AAA TCT TAC	1200
Glu Asn Gly Lys Leu Ser Glu Gly Val Thr Ile Ser Tyr Lys Ser Tyr	
365                      370                      375                      380	
TGC AAG AAC GGG GTG AAT GGA ACA GGG GAA AAT GGA AGA AAA TGT TCC	1248
Cys Lys Asn Gly Val Asn Gly Thr Gly Glu Asn Gly Arg Lys Cys Ser	
385                      390                      395	
AAT ATT TCC ATT GGA GAT GAG GTT CAA TTT GAA ATT AGC ATA ACT TCA	1296
Asn Ile Ser Ile Gly Asp Glu Val Gln Phe Glu Ile Ser Ile Thr Ser	
400                      405                      410	
AAT AAG TGT CCA AAA AAG GAT TCT GAC AGC TTT AAA ATT AGG CCT CTG	1344
Asn Lys Cys Pro Lys Lys Asp Ser Asp Ser Phe Lys Ile Arg Pro Leu	
415                      420                      425	
GGC TTT ACG GAG GAA GTA GAG GTT ATT CTT CAG TAC ATC TGT GAA TGT	1392
Gly Phe Thr Glu Glu Val Glu Val Ile Leu Gln Tyr Ile Cys Glu Cys	
430                      435                      440	
GAA TGC CAA AGC GAA GGC ATC CCT GAA AGT CCC AAG TGT CAT GAA GGA	1440
Glu Cys Gln Ser Glu Gly Ile Pro Glu Ser Pro Lys Cys His Glu Gly	
445                      450                      455                      460	
AAT GGG ACA TTT GAG TGT GGC GCG TGC AGG TGC AAT GAA GGG CGT GTT	1488
Asn Gly Thr Phe Glu Cys Gly Ala Cys Arg Cys Asn Glu Gly Arg Val	
465                      470                      475	
GGT AGA CAT TGT GAA TGC AGC ACA GAT GAA GTT AAC AGT GAA GAC ATG	1536
Gly Arg His Cys Glu Cys Ser Thr Asp Glu Val Asn Ser Glu Asp Met	
480                      485                      490	
GAT GCT TAC TGC AGG AAA GAA AAC AGT TCA GAA ATC TGC AGT AAC AAT	1584
Asp Ala Tyr Cys Arg Lys Glu Asn Ser Ser Glu Ile Cys Ser Asn Asn	
495                      500                      505	
GGA GAG TGC GTC TGC GGA CAG TGT GTT TGT AGG AAG AGG GAT AAT ACA	1632
Gly Glu Cys Val Cys Gly Gln Cys Val Cys Arg Lys Arg Asp Asn Thr	
510                      515                      520	

AAT GAA ATT TAT TCT GGC AAA TTC TGC GAG TGT GAT AAT TTC AAC TGT	1680
Asn Glu Ile Tyr Ser Gly Lys Phe Cys Glu Cys Asp Asn Phe Asn Cys	
525 530 535 540	
GAT AGA TCC AAT GGC TTA ATT TGT GGA GGA AAT GGT GTT TGC AAG TGT	1728
Asp Arg Ser Asn Gly Leu Ile Cys Gly Gly Asn Gly Val Cys Lys Cys	
545 550 555	
CGT GTG TGT GAG TGC AAC CCC AAC TAC ACT GGC AGT GCA TGT GAC TGT	1776
Arg Val Cys Glu Cys Asn Pro Asn Tyr Thr Gly Ser Ala Cys Asp Cys	
560 565 570	
TCT TTG GAT ACT AGT ACT TGT GAA GCC AGC AAC GGA CAG ATC TGC AAT	1824
Ser Leu Asp Thr Ser Thr Cys Glu Ala Ser Asn Gly Gln Ile Cys Asn	
575 580 585	
GGC CGG GGC ATC TGC GAG TGT GGT GTC TGT AAG TGT ACA GAT CCG AAG	1872
Gly Arg Gly Ile Cys Glu Cys Gly Val Cys Lys Cys Thr Asp Pro Lys	
590 595 600	
TTT CAA GGG CAA ACG TGT GAG ATG TGT CAG ACC TGC CTT GGT GTC TGT	1920
Phe Gln Gly Gln Thr Cys Glu Met Cys Gln Thr Cys Leu Gly Val Cys	
605 610 615 620	
GCT GAG CAT AAA GAA TGT GTT CAG TGC AGA GCC TTC AAT AAA GGA GAA	1968
Ala Glu His Lys Glu Cys Val Gln Cys Arg Ala Phe Asn Lys Gly Glu	
625 630 635	
AAG AAA GAC ACA TGC ACA CAG GAA TGT TCC TAT TTT AAC ATT ACC AAG	2016
Lys Lys Asp Thr Cys Thr Gln Glu Cys Ser Tyr Phe Asn Ile Thr Lys	
640 645 650	
GTA GAA AGT CGG GAC AAA TTA CCC CAG CCG GTC CAA CCT GAT CCT GTG	2064
Val Glu Ser Arg Asp Lys Leu Pro Gln Pro Val Gln Pro Asp Pro Val	
655 660 665	
TCC CAT TGT AAG GAG AAG GAT GTT GAC GAC TGT TGG TTC TAT TTT ACG	2112
Ser His Cys Lys Glu Lys Asp Val Asp Asp Cys Trp Phe Tyr Phe Thr	
670 675 680	

TAT TCA GTG AAT GGG AAC AAC GAG GTC ATG GTT CAT GTT GTG GAG AAT 2160  
Tyr Ser Val Asn Gly Asn Asn Glu Val Met Val His Val Val Glu Asn  
685 690 695 700  
CCA GAG TGT CCC ACT GGT CCA GAG GAT CCC GAG CTGCTGGAAG CAGGCTCAGC 2213  
Pro Glu Cys Pro Thr Gly Pro Glu Asp Pro Glu  
705 710  
GCTCCTGCCT GGACGCATCC CGGCTATGCA GCCCCAGTCC AGGGCAGCAA GGCAGGCCCC 2273  
GTCTGCCTCT TCACCCGGAG CCTCTGCCCC GCCCCTCAT GCTCAGGGAG AGGGTCTTCT 2333  
GGCTTTTTCC CAGGCTCTGG GCAGGCACAG GCTAGGTGCC CCTAAGCCAG GCCCTGCACA 2393  
CAAAGGGGCA GGTGCTGGGC TCAGACCTGC CAAGAGCCAT ATCCGGGAGG ACCCTGCCCC 2453  
TGACCTAAGC CCACCCAAA GGCCAACTC TCCACTCCCT CAGCTCGGAC ACCTTCTCTC 2513  
CTCCCAGATT CCAGTAACTC CCAATCTTCT CTCTGCA GAG CCC AAA TCT TGT GAC 2568  
Glu Pro Lys Ser Cys Asp  
715  
AAA ACT CAC ACA TGC CCA CCG TGC CCA GGTAAGCCAG CCCAGGCCTC 2615  
Lys Thr His Thr Cys Pro Pro Cys Pro  
720 725  
GCCCTCCAGC TCAAGGCGGG ACAGGTGCCC TAGAGTAGCC TGCATCCAGG GACAGGCCCC 2675  
AGCCGGGTGC TGACACGTCC ACCTCCATCT CTTCCTCA GCA CCT GAA CTC CTG 2728  
Ala Pro Glu Leu Leu  
730  
GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC 2776  
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu  
735 740 745  
ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC 2824  
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser  
750 755 760  
CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG 2872  
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu  
765 770 775

[illegible]

87

Topology: Linear

Kind of sequence: Other nucleic acid, synthetic DNA

Sequence

CCTCTAGACG GCCGTCGCAC TCATTTA

27

Sequence No. 6

Length of sequence: 73

Type of sequence: Nucleic acid

Number of strands: Single

Topology: Linear

Kind of sequence: Other nucleic acid, synthetic DNA

Sequence

CTAGACCACC ATGTTCCCA CCGAGAGCGC ATGGCTTGGG AAGCGAGGCG CGAACCCGGG  
CCCCGGAGCT GCA

73

Sequence No. 7

Length of sequence: 65

Type of sequence: Nucleic acid

Number of strands: Single

Topology: Linear

Kind of sequence: Other nucleic acid, synthetic DNA

Sequence

GCTTCGGGGC CCGGGTTCGC GCCTCGCTTC CCAAGCCATG CGCTCTCGGT GGGGAACATG  
GTGGT

65

Sequence No. 8

Length of sequence: 51

Type of sequence: Nucleic acid

Number of strands: Single

Topology: Linear

Kind of sequence: Other nucleic acid, synthetic DNA  
Sequence

CTCCGGGAGA CGGTGATGCT GTTGCTGTGC CTGGGGGTCC CGACCGGCAG G

51

Sequence No. 9

Length of sequence: 55

Type of sequence: Nucleic acid

Number of strands: Single

Topology: Straight chain

Kind of sequence: Other nucleic acid, synthetic DNA  
Sequence

CCTGCCGGTC GGGACCCCCA GGCACAGCAA CAGCATCACC GTCTCCCGGA GTCGA

55

Sequence No. 10

Length of sequence: 37

Type of sequence: Nucleic acid

Number of strands: Single

Topology: Linear

Kind of sequence: Other nucleic acid, synthetic DNA  
Sequence

CACTGCAGGC AGGCCTTACA ACGTGGACAC TGAGAGC

37

Sequence No. 11

Length of sequence: 22

Type of sequence: Nucleic acid

Number of strands: Single

Topology: Linear

Kind of sequence: Other nucleic acid, synthetic DNA  
Sequence

GCAGAAACCT GTAAATCAGC AG

22

Sequence No. 12

Length of sequence: 22

Type of sequence: Nucleic acid

Number of strands: Single

Topology: Linear

Kind of sequence: Other nucleic acid, synthetic DNA  
Sequence

GCATTTATGC GGAAAGATGT GC

22

Sequence No. 13

Length of sequence: 29

Type of sequence: Nucleic acid

Number of strands: Single

Topology: Linear

Kind of sequence: Other nucleic acid, synthetic DNA  
Sequence

CGGGATCCGT GAAATAACGT TTGGGTCTT

29

Sequence No. 14

Length of sequence: 22

Type of sequence: Nucleic acid

Number of strands: Single

Topology: Linear

Kind of sequence: Other nucleic acid, synthetic DNA  
Sequence

GCGGAAAAGA TGAATTTACA AC

22

Sequence No. 15

Length of sequence: 27

Type of sequence: Nucleic acid

Number of strands: Single

Topology: Linear

Kind of sequence: Other nucleic acid, synthetic DNA  
Sequence

GTGGGATCCT CTGGACCACT GGGACAC

27

Sequence No. 16

Length of sequence: 10

Type of sequence: Amino acid

Topology: Linear

Kind of sequence: Peptide

Sequence

Gly Pro Glu Ile Leu Asp Val Pro Ser Thr

1 5 10

Sequence No. 17

Length of sequence: 10

Type of sequence: Amino acid

Topology: Linear

Kind of sequence: Peptide

Sequence

Gly Pro Glu Ile Leu Glu Val Pro Ser Thr

1 5 10

Sequence No. 18

Length of sequence: 6

Type of sequence: Amino acid

Topology: Linear

Kind of sequence: Peptide

# Sequence

Gly Arg Gly Asp Ser Pro

1 5

Sequence No. 19

Length of sequence: 4675

Type of sequence: Nucleic acid

## Sequence

ATG GGG CCA GAA CGG ACA GGG GCC GCG CCG CTG CCG CTG CTG CTG GTG	48
Met Gly Pro Glu Arg Thr Gly Ala Ala Pro Leu Pro Leu Leu Leu Val	
-25 -20 -15	
TTA GCG CTC AGT CAA GGC ATT TTA AAT TGT TGT TTG GCC TAC AAT GTT	96
Leu Ala Leu Ser Gln Gly Ile Leu Asn Cys Cys Leu Ala Tyr Asn Val	
-10 -5 1	
GGT CTC CCA GAA GCA AAA ATA TTT TCC GGT CCT TCA AGT GAA CAG TTT	144
Gly Leu Pro Glu Ala Lys Ile Phe Ser Gly Pro Ser Ser Glu Gln Phe	
5 10 15	
GGG TAT GCA GTG CAG CAG TTT ATA AAT CCA AAA GGC AAC TGG TTA CTG	192
Gly Tyr Ala Val Gln Gln Phe Ile Asn Pro Lys Gly Asn Trp Leu Leu	
20 25 30 35	
GTT GGT TCA CCC TGG AGT GGC TTT CCT GAG AAC CCA ATG GGA GAT GTG	240
Val Gly Ser Pro Trp Ser Gly Phe Pro Glu Asn Arg Met Gly Asp Val	
40 45 50	
TAT AAA TGT CCT GTT GAC CTA TCC ACT GCC ACA TGT GAA AAA CTA AAT	288
Tyr Lys Cys Pro Val Asp Leu Ser Thr Ala Thr Cys Glu Lys Leu Asn	
55 60 65	
TTG CAA ACT TCA ACA AGC ATT CCA AAT GTT ACT GAG ATG AAA ACC AAC	336
Leu Gln Thr Ser Thr Ser Ile Pro Asn Val Thr Glu Met Lys Thr Asn	
70 75 80	

ATG AGC CTC GGC TTG ATC CTC ACC AGG AAC ATG GGA ACT GGA GGT TTT	384
Met Ser Leu Gly Leu Ile Leu Thr Arg Asn Met Gly Thr Gly Gly Phe	
85 90 95	
CTC ACA TGT GGT CCT CTG TGG GCA CAG CAA TGT GGG AAT CAG TAT TAC	432
Leu Thr Cys Gly Pro Leu Trp Ala Gln Gln Cys Gly Asn Gln Tyr Tyr	
100 105 110 115	
ACA ACG GGT GTG TGT TCT GAC ATC AGT CCT GAT TTT CAG CTC TCA GCC	480
Thr Thr Gly Val Cys Ser Asp Ile Ser Pro Asp Phe Gln Leu Ser Ala	
120 125 130	
AGC TTC TCA CCT GCA ACT CAG CCC TGC CCT TCC CTC ATA GAT GTT GTG	528
Ser Phe Ser Pro Ala Thr Gln Pro Cys Pro Ser Leu Ile Asp Val Val	
135 140 145	
GTT GTG TGT GAT GAA TCA AAT AGT ATT TAT CCT TGG GAT GCA GTA AAG	576
Val Val Cys Asp Glu Ser Asn Ser Ile Tyr Pro Trp Asp Ala Val Lys	
150 155 160	
AAT TTT TTG GAA AAA TTT GTA CAA GGC CTT GAT ATA GGC CCC ACA AAG	624
Asn Phe Leu Glu Lys Phe Val Gln Gly Leu Asp Ile Gly Pro Thr Lys	
165 170 175	
ACA CAG GTG GGG TTA ATT CAG TAT GCC AAT AAT CCA AGA GTT GTG TTT	672
Thr Gln Val Gly Leu Ile Gln Tyr Ala Asn Asn Pro Arg Val Val Phe	
180 185 190 195	
AAC TTG AAC ACA TAT AAA ACC AAA GAA GAA ATG ATT GTA GCA ACA TCC	720
Asn Leu Asn Thr Tyr Lys Thr Lys Glu Glu Met Ile Val Ala Thr Ser	
200 205 210	
CAG ACA TCC CAA TAT GGT GGG GAC CTC ACA AAC ACA TTC GGA GCA ATT	768
Gln Thr Ser Gln Tyr Gly Gly Asp Leu Thr Asn Thr Phe Gly Ala Ile	
215 220 225	
CAA TAT GCA AGA AAA TAT GCC TAT TCA GCA GCT TCT GGT GGG CGA CGA	816
Gln Tyr Ala Arg Lys Tyr Ala Tyr Ser Ala Ala Ser Gly Gly Arg Arg	
230 235 240	

AGT GCT ACG AAA GTA ATG GTA GTT GTA ACT GAC GGT GAA TCA CAT GAT	864
Ser Ala Thr Lys Val Met Val Val Val Thr Asp Gly Glu Ser His Asp	
245 250 255	
GCT TCA ATG TTG AAA GCT GTG ATT GAT CAA TGC AAC CAT GAC AAT ATA	912
Gly Ser Met Leu Lys Ala Val Ile Asp Gln Cys Asn His Asp Asn Ile	
260 265 270 275	
CTG AGG TTT GGC ATA GCA GTT CTT GGG TAC TTA AAC AGA AAC GCC CTT	960
Leu Arg Phe Gly Ile Ala Val Leu Gly Tyr Leu Asn Arg Asn Ala Leu	
280 285 290	
GAT ACT AAA AAT TTA ATA AAA GAA ATA AAA GCG ATC GCT AGT ATT CCA	1008
Asp Thr Lys Asn Leu Ile Lys Glu Ile Lys Ala Ile Ala Ser Ile Pro	
295 300 305	
ACA GAA AGA TAC TTT TTC AAT GTG TCT GAT GAA GCA GCT CTA CTA GAA	1056
Thr Glu Arg Tyr Phe Phe Asn Val Ser Asp Glu Ala Ala Leu Leu Glu	
310 315 320	
AAG GCT GGG ACA TTA GGA GAA CAA ATT TTC AGC ATT GAA GGT ACT GTT	1104
Lys Ala Gly Thr Leu Gly Glu Gln Ile Phe Ser Ile Glu Gly Thr Val	
325 330 335	
CAA GGA GGA GAC AAC TTT CAG ATG GAA ATG TCA CAA GTG GGA TTC AGT	1152
Gln Gly Gly Asp Asn Phe Gln Met Glu Met Ser Gln Val Gly Phe Ser	
340 345 350 355	
GCA GAT TAC TCT TCT CAA AAT GAT ATT CTG ATG CTG GGT GCA GTG GGA	1200
Ala Asp Tyr Ser Ser Gln Asn Asp Ile Leu Met Leu Gly Ala Val Gly	
360 365 370	
GCT TTT GGC TGG AGT GGG ACC ATT GTC CAG AAG ACA TCT CAT GGC CAT	1248
Ala Phe Gly Trp Ser Gly Thr Ile Val Gln Lys Thr Ser His Gly His	
375 380 385	
TTG ATC TTT CCT AAA CAA GCC TTT GAC CAA ATT CTG CAG GAC AGA AAT	1296
Leu Ile Phe Pro Lys Gln Ala Phe Asp Gln Ile Leu Gln Asp Arg Asn	
390 395 400	

CAC AGT TCA TAT TTA GGT TAC TCT GTG GCT GCA ATT TCT ACT GGA GAA	1344
His Ser Ser Tyr Leu Gly Tyr Ser Val Ala Ala Ile Ser Thr Gly Glu	
405 410 415	
AGC ACT CAC TTT GTT GCT GGT GCT CCT CGG GCA AAT TAT ACC GGC CAG	1392
Ser Thr His Phe Val Ala Gly Ala Pro Arg Ala Asn Tyr Thr Gly Gln	
420 425 430 435	
ATA GTG CTA TAT AGT GTG AAT GAG AAT GGC AAT ATC ACG GTT ATT CAG	1440
Ile Val Leu Tyr Ser Val Asn Glu Asn Gly Asn Ile Thr Val Ile Gln	
440 445 450	
GCT CAC CGA GGT GAC CAG ATT GGC TCC TAT TTT GGT AGT GTG CTG TGT	1488
Ala His Arg Gly Asp Gln Ile Gly Ser Tyr Phe Gly Ser Val Leu Cys	
455 460 465	
TCA GTT GAT GTG GAT AAA GAC ACC ATT ACA GAC GTG CTC TTG GTA GGT	1536
Ser Val Asp Val Asp Lys Asp Thr Ile Thr Asp Val Leu Leu Val Gly	
470 475 480	
GCA CCA ATG TAC ATG AGT GAC CTA AAG AAA GAG GAA GGA AGA GTC TAC	1584
Ala Pro Met Tyr Met Ser Asp Leu Lys Lys Glu Glu Gly Arg Val Tyr	
485 490 495	
CTG TTT ACT ATC AAA AAG GGC ATT TTG GGT CAG CAC CAA TTT CTT GAA	1632
Leu Phe Thr Ile Lys Lys Gly Ile Leu Gly Gln His Gln Phe Leu Glu	
500 505 510 515	
GGC CCC GAG GGC ATT GAA AAC ACT CGA TTT GGT TCA GCA ATT GCA GCT	1680
Gly Pro Glu Gly Ile Glu Asn Thr Arg Phe Gly Ser Ala Ile Ala Ala	
520 525 530	
CTT TCA GAC ATC AAC ATG GAT GGC TTT AAT GAT GTG ATT GTT GGT TCA	1728
Leu Ser Asp Ile Asn Met Asp Gly Phe Asn Asp Val Ile Val Gly Ser	
535 540 545	
CCA CTA GAA AAT CAG AAT TCT GGA GCT GTA TAC ATT TAC AAT GGT CAT	1776
Pro Leu Glu Asn Gln Asn Ser Gly Ala Val Tyr Ile Tyr Asn Gly His	
550 555 560	

CAG GGC ACT ATC CGC ACA AAG TAT TCC CAG AAA ATC TTG GGA TCC GAT	1824
Gln Gly Thr Ile Arg Thr Lys Tyr Ser Gln Lys Ile Leu Gly Ser Asp	
565 570 575	
GGA GCC TTT AGG AGC CAT CTC CAG TAC TTT GGG AGG TCC TTC GAT GGC	1872
Gly Ala Phe Arg Ser His Leu Gln Tyr Phe Gly Arg Ser Leu Asp Gly	
580 585 590 595	
TAT GGA GAT TTA AAT GGG GAT TCC ATC ACC GAT GTG TCT ATT GGT GCC	1920
Tyr Gly Asp Leu Asn Gly Asp Ser Ile Thr Asp Val Ser Ile Gly Ala	
600 605 610	
TTT GGA CAA GTG GTT CAA CTC TGG TCA CAA AGT ATT GCT GAT GTA GCT	1968
Phe Gly Gln Val Val Gln Leu Trp Ser Gln Ser Ile Ala Asp Val Ala	
615 620 625	
ATA GAA GCT TCA TTC ACA CCA GAA AAA ATC ACT TTG GTC AAC AAG AAT	2016
Ile Glu Ala Ser Phe Thr Pro Glu Lys Ile Thr Leu Val Asn Lys Asn	
630 635 640	
GCT CAG ATA ATT CTC AAA CTC TGC TTC AGT GCA AAG TTC AGA CCT ACT	2064
Ala Gln Ile Ile Leu Lys Leu Cys Phe Ser Ala Lys Phe Arg Pro Thr	
645 650 655	
AAG CAA AAC AAT CAA GTG GCC ATT GTA TAT AAC ATC ACA CTT GAT GCA	2112
Lys Gln Asn Asn Gln Val Ala Ile Val Tyr Asn Ile Thr Leu Asp Ala	
660 665 670 675	
GAT GGA TTT TCA TCC AGA GTA ACC TCC AGG GGG TTA TTT AAA GAA AAC	2160
Asp Gly Phe Ser Ser Arg Val Thr Ser Arg Gly Leu Phe Lys Glu Asn	
680 685 690	
AAT GAA AGG TGC CTG CAG AAG AAT ATG GTA GTA AAT CAA GCA CAG AGT	2208
Asn Glu Arg Cys Leu Gln Lys Asn Met Val Val Asn Gln Ala Gln Ser	
695 700 705	
TGC CCC GAG CAC ATC ATT TAT ATA CAG GAG CCC TCT GAT GTT GTC AAC	2256
Cys Pro Glu His Ile Ile Tyr Ile Gln Glu Pro Ser Asp Val Val Asn	
710 715 720	

TCT TTG GAT TTG CGT GTG GAC ATC AGT CTG GAA AAC CCT GGC ACT AGC	2304
Ser Leu Asp Leu Arg Val Asp Ile Ser Leu Glu Asn Pro Gly Thr Ser	
725 730 735	
CCT GCC CTT GAA GCC TAT TCT GAG ACT GCC AAG GTC TTC AGT ATT CCT	2352
Pro Ala Leu Glu Ala Tyr Ser Glu Thr Ala Lys Val Phe Ser Ile Pro	
740 745 750 755	
TTC CAC AAA GAC TGT GGT GAG GAT GGA CTT TGC ATT TCT GAT CTA GTC	2400
Phe His Lys Asp Cys Gly Glu Asp Gly Leu Cys Ile Ser Asp Leu Val	
760 765 770	
CTA GAT GTC CGA CAA ATA CCA GCT GCT CAA GAA CAA CCC TTT ATT GTC	2448
Leu Asp Val Arg Gln Ile Pro Ala Ala Gln Glu Gln Pro Phe Ile Val	
775 780 785	
AGC AAC CAA AAC AAA AGG TTA ACA TTT TCA GTA ACA CTG AAA AAT AAA	2496
Ser Asn Gln Asn Lys Arg Leu Thr Phe Ser Val Thr Leu Lys Asn Lys	
790 795 800	
AGG GAA AGT GCA TAC AAC ACT GGA ATT GTT GTT GAT TTT TCA GAA AAC	2544
Arg Glu Ser Ala Tyr Asn Thr Gly Ile Val Val Asp Phe Ser Glu Asn	
805 810 815	
TTG TTT TTT GCA TCA TTC TCC CTA CCG GTT GAT GGG ACA GAA GTA ACA	2592
Leu Phe Phe Ala Ser Phe Ser Leu Pro Val Asp Gly Thr Glu Val Thr	
820 825 830 835	
TGC CAG GTG GCT GCA TCT CAG AAG TCT GTT GCC TGC GAT GTA GGC TAC	2640
Cys Gln Val Ala Ala Ser Gln Lys Ser Val Ala Cys Asp Val Gly Tyr	
840 845 850	
CCT GCT TTA AAG AGA GAA CAA CAG GTG ACT TTT ACT ATT AAC TTT GAC	2688
Pro Ala Leu Lys Arg Glu Gln Gln Val Thr Phe Thr Ile Asn Phe Asp	
855 860 865	
TTC AAT CTT CAA AAC CTT CAG AAT CAG GCG TCT CTC ACT TTC CAA GCC	2736
Phe Asn Leu Gln Asn Leu Gln Asn Gln Ala Ser Leu Ser Phe Gln Ala	
870 875 880	

TTA AGT GAA AGC CAA GAA GAA AAC AAG GCT GAT AAT TTG GTC AAC CTC	2784
Leu Ser Glu Ser Gln Glu Glu Asn Lys Ala Asp Asn Leu Val Asn Leu	
885 890 895	
AAA ATT CCT CTC CTG TAT GAT GCT GAA ATT CAC TTA ACA AGA TCT ACC	2832
Lys Ile Pro Leu Leu Tyr Asp Ala Glu Ile His Leu Thr Arg Ser Thr	
900 905 910 915	
AAC ATA AAT TTT TAT GAA ATC TCT TCG GAT GGG AAT GTT CCT TCA ATC	2880
Asn Ile Asn Phe Tyr Glu Ile Ser Ser Asp Gly Asn Val Pro Ser Ile	
920 925 930	
GTG CAC AGT TTT GAA GAT GTT GGT CCA AAA TTC ATC TTC TCC CTG AAG	2928
Val His Ser Phe Glu Asp Val Gly Pro Lys Phe Ile Phe Ser Leu Lys	
935 940 945	
GTA ACA ACA GGA AGT GTT CCA GTA AGC ATG GCA ACT GTA ATC ATC CAC	2976
Val Thr Thr Gly Ser Val Pro Val Ser Met Ala Thr Val Ile Ile His	
950 955 960	
ATC CCT CAG TAT ACC AAA GAA AAG AAC CCA CTG ATG TAC CTA ACT GGG	3024
Ile Pro Gln Tyr Thr Lys Glu Lys Asn Pro Leu Met Tyr Leu Thr Gly	
965 970 975	
GTG CAA ACA GAC AAG GCT GGT GAC ATC AGT TGT AAT GCA GAT ATC AAT	3072
Val Gln Thr Asp Lys Ala Gly Asp Ile Ser Cys Asn Ala Asp Ile Asn	
980 985 990 995	
CCA CTG AAA ATA GGA CAA ACA TCT TCT TCT GTA TCT TTC AAA AGT GAA	3120
Pro Leu Lys Ile Gly Gln Thr Ser Ser Ser Val Ser Phe Lys Ser Glu	
1000 1005 1010	
AAT TTC AGG CAC ACC AAA GAA TTG AAC TGC AGA ACT GCT TCC TGT AGT	3168
Asn Phe Arg His Thr Lys Glu Leu Asn Cys Arg Thr Ala Ser Cys Ser	
1015 1020 1025	
AAT GTT ACC TGC TCG TTG AAA GAC GTT CAC ATG AAA GGA GAA TAC TTT	3216
Asn Val Thr Cys Trp Leu Lys Asp Val His Met Lys Gly Glu Tyr Phe	
1030 1035 1040	

GTT AAT GTG ACT ACC AGA ATT TGG AAC GGG ACT TTC GCA TCA TCA ACG	3264
Val Asn Val Thr Thr Arg Ile Trp Asn Gly Thr Phe Ala Ser Ser Thr	
1045	1050
TTC CAG ACA GTA CAG CTA ACG GCA GCT GCA GAA ATC AAC ACC TAT AAC	3312
Phe Gln Thr Val Gln Leu Thr Ala Ala Ala Glu Ile Asn Thr Tyr Asn	
1060	1065
CCT GAG ATA TAT GTG ATT GAA GAT AAC ACT GTT ACG ATT CCC CTG ATG	3360
Pro Glu Ile Tyr Val Ile Glu Asp Asn Thr Val Thr Ile Pro Leu Met	
1080	1085
ATA ATG AAA CCT GAT GAG AAA GCC GAA GTA CCA ACA GAT CCC GAG	3405
Ile Met Lys Pro Asp Glu Lys Ala Glu Val Pro Thr Asp Pro Glu	
1095	1100
CTGCTGGAAG CAGGCTCAGC GCTCCTGCCT GGACGCATCC CGGCTATGCA GCCCCAGTCC	3465
AGGGCAGCAA GGCAGGCCCC GTCTGCCTCT TCACCCGGAG CCTCTGCCCC CCCCCTCAT	3525
GCTCAGGGAG AGGGTCTTCT GGCTTTTTTC CAGGCTCTGG GCAGGCACAG GCTAGGTGCC	3585
CCTAACCCAG GCCCTGCACA CAAAGGGGCA GGTGCTGGGC TCAGACCTGC CAAGAGCCAT	3645
ATCCGGGAGG ACCCTGCCCC TGACCTAAGC CCACCCCAAA GGCCAAACTC TCCACTCCCT	3705
CAGCTCGGAC ACCTTCTCTC CTCCCAGATT CCAGTAACTC CCAATCTTCT CTCTGCA	3762
GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA	3807
Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro	
1110	1115
GGTAAGCCAG CCCAGGCCTC GCCCTCCAGC TCAAGGCGGG ACAGGTGCCC TAGAGTAGCC	3867
TGCATCCAGG GACAGGCCCC AGCCGGGTGC TGACACGTCC ACCTCCATCT CTTCCTCA	3925
GCA CCT GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA	3973
Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys	
1125	1130
CCC AAG GAC ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG	4021
Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val	
1140	1145
	1150

GTG GTG GAC GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC	4069
Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr	
1155 1160 1165	
GTG GAC GGC GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG	4117
Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu	
1170 1175 1180 1185	
CAG TAC AAC AGC ACG TAC CGG GTG GTC AGC GTC CTC ACC GTC CTG CAC	4165
Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His	
1190 1195 1200	
CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA	4213
Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys	
1205 1210 1215	
GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA	4255
Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys	
1220 1225 1230	
GGTGGGACCC GTGGGGTGCG AGGGCCACAT GGACAGAGGC CGGCTCGGCC CACCCTCTGC	4315
CCTGAGAGTG ACCGCTGTAC CAACCTCTGT CCTACA GGG CAG CCC CGA GAA CCA	4369
Gly Gln Pro Arg Glu Pro	
1235	
CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG ACC AAG AAC CAG	4417
Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln	
1240 1245 1250	
GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC GCC	4465
Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala	
1255 1260 1265	
GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG	4513
Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr	
1270 1275 1280 1285	

CCT CCC GTG CTG GAT TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC 4561  
 Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu

1290 1295 1300

ACC GTG GAC AAG AGC AGG TGG CAG CAG GCG AAC GTC TTC TCA TGC TCC 4609  
 Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser

1305 1310 1315

GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC 4657  
 Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser

1320 1325 1330

CTG TCT CCG GGT AAA TGA 4675  
 Leu Ser Pro Gly Lys

1335

Sequence No. 20

Length of sequence: 27

Type of sequence: Nucleic acid

Number of strands: Single

Topology: Linear

Kind of sequence: Other nucleic acid, synthetic DNA

Sequence

GCTCGAGCAA ACCCAGCGCA ACTACGG 27

Sequence No. 21

Length of sequence: 21

Type of sequence: Nucleic acid

Number of strands: Single

Topology: Linear

Kind of sequence: Other nucleic acid, synthetic DNA

Sequence

ATAGTGGCCT GATGACCATT G 21

Sequence No. 22

Length of sequence: 22

Type of sequence: Nucleic acid

Number of strands: Single

Topology: Linear

Kind of sequence: Other nucleic acid, synthetic DNA

Sequence

GATGGCTTTA ATGATGTGAT TG

22

Sequence No. 23

Length of sequence: 21

Type of sequence: Nucleic acid

Number of strands: Single

Topology: Linear

Kind of sequence: Other nucleic acid, synthetic DNA

Sequence

TGTTGGTACT TCGGCTTTCT C

21

Sequence No. 24

Length of sequence: 8

Type of sequence: Amino acid

Topology: Circular

Kind of sequence: Peptide

Sequence

Cys Ile Pro Glu Leu Ile Val Cys

1

5

Sequence No. 25

Length of sequence: 8

Type of sequence: Amino acid

Topology: Circular

Kind of sequence: Peptide

Sequence

Cys Met Arg Tyr Thr Ser Ala Cys

1

5

Sequence No. 26

Length of sequence: 8

Type of sequence: Amino acid

Topology: Circular

Kind of sequence: Peptide

Sequence

Cys Glu Trp Met Lys Arg Phe Cys

1

5

Sequence No. 27

Length of sequence: 8

Type of sequence: Amino acid

Topology: Circular

Kind of sequence: Peptide

Sequence

Cys Tyr Thr Thr Arg Leu Lys Cys

1

5

Sequence No. 28

Length of sequence: 8

Type of sequence: Amino acid

Topology: Circular

Kind of sequence: Peptide

Sequence

Cys Leu Arg Tyr Ser Val Pro Cys

1 5

Sequence No. 29

Length of sequence: 8

Type of sequence: Amino acid

Topology: Circular

Kind of sequence: Peptide

Sequence

Cys Ile Val Asn Arg Leu Gly Cys

1 5

Sequence No. 30

Length of sequence: 8

Type of sequence: Amino acid

Topology: Circular

Kind of sequence: Peptide

Sequence

Cys Gly Leu Gln Ala Leu Pro Cys

1 5

Sequence No. 31

Length of sequence: 8

Type of sequence: Amino acid

Topology: Circular

Kind of sequence: Peptide

Sequence

Cys Lys Leu Lys Gly Thr Met Cys

1 5

Claims

1. A chimeric protein comprising the  $\alpha$  chain or  $\beta$  chain of an integrin and the heavy chain or light chain of an immunoglobulin.
2. A chimeric protein heterodimer complex, characterized in that a chimeric protein stated in claim 1 comprising the  $\alpha$  chain of an integrin and the heavy chain or light chain of an immunoglobulin and a chimeric protein stated in claim 1 comprising the  $\beta$  chain of the integrin and the heavy chain or light chain of the immunoglobulin are associated with each other.
3. A chimeric protein heterodimer complex, according to claim 2, wherein the chimeric proteins stated in claim 1 are associated with each other in any of the following combinations (1), (2) and (3):
  - (1) An  $\alpha$  chain-immunoglobulin heavy chain- $\beta$  chain-immunoglobulin heavy chain chimeric protein heterodimer complex, in which a chimeric protein comprising the  $\alpha$  chain of an integrin and the heavy chain of an immunoglobulin and a chimeric protein comprising the  $\beta$  chain of the integrin and the heavy chain of the immunoglobulin are associated with each other.
  - (2) An  $\alpha$  chain-immunoglobulin heavy chain- $\beta$  chain-immunoglobulin light chain chimeric protein heterodimer complex, in which a chimeric protein comprising the  $\alpha$  chain of

an integrin and the heavy chain of an immunoglobulin and a chimeric protein comprising the  $\beta$  chain of the integrin and the light chain of the immunoglobulin are associated with each other.

(3) An  $\alpha$  chain-immunoglobulin light chain- $\beta$  chain-immunoglobulin heavy chain chimeric protein heterodimer complex, in which a chimeric protein comprising the  $\alpha$  chain of an integrin and the light chain of an immunoglobulin and a chimeric protein comprising of the  $\beta$  chain of the integrin and the heavy chain of the immunoglobulin are associated with each other.

4. A chimeric protein or a chimeric protein heterodimer complex, according to any one of claims 1 through 3, wherein the  $\alpha$  chain of an integrin is  $\alpha 1, \alpha 2, \alpha 3, \alpha 4, \alpha 5, \alpha 6, \alpha 7, \alpha 8, \alpha 9, \alpha v, \alpha L, \alpha M, \alpha X, \alpha 11b$  or  $\alpha E$ .

5. A chimeric protein or a chimeric protein heterodimer complex, according to any one of claims 1 through 3, wherein the  $\beta$  chain of an integrin is  $\beta 1, \beta 2, \beta 3, \beta 4, \beta 5, \beta 6, \beta 7$  or  $\beta 8$ .

6. A chimeric protein heterodimer complex, according to claim 2 or 3, wherein the  $\alpha$  chain of an integrin is  $\alpha 4$  or  $\alpha 2$  and the  $\beta$  chain is  $\beta 1$ .

7. A chimeric protein or a chimeric protein heterodimer complex, according to any one of claims 1 through 3, wherein the chimeric protein comprising the  $\alpha 4$  of an integrin and the

heavy chain of an immunoglobulin is identified as the amino acid sequence of sequence No. 1.

8. A chimeric protein or a chimeric protein heterodimer complex, according to any one of claims 1 through 3, wherein the chimeric protein comprising the  $\alpha 2$  of an integrin and the heavy chain of an immunoglobulin is identified as the amino acid sequence of sequence No. 19.

9. A chimeric protein or a chimeric protein heterodimer complex, according to any one of claims 1 through 3, wherein the chimeric protein comprising the  $\beta 1$  of an integrin and the heavy chain of an immunoglobulin is identified as the amino acid sequence of sequence No. 2.

10. A DNA coding for a chimeric protein stated in claim 1.

11. A DNA coding for a chimeric protein stated in claim 1, wherein the  $\alpha$  chain of an integrin is  $\alpha 1, \alpha 2, \alpha 3, \alpha 4, \alpha 5, \alpha 6, \alpha 7, \alpha 8, \alpha 9, \alpha v, \alpha L, \alpha M, \alpha X, \alpha 11b$  or  $\alpha E$ .

12. A DNA coding for a chimeric protein stated in claim 1, wherein the  $\beta$  chain of an integrin is  $\beta 1, \beta 2, \beta 3, \beta 4, \beta 5, \beta 6, \beta 7$  or  $\beta 8$ .

13. A DNA, according to claim 11, which is identified as the nucleotide sequence of sequence No. 1 or 19.

14. A DNA, according to claim 12, which is identified as the nucleotide sequence of sequence No. 2.

15. A recombinant vector, wherein a DNA stated in claim 10 is functionally linked to an expression control sequence.

16. A recombinant vector, wherein a DNA stated in claim 11 is functionally linked to an expression control sequence.
17. A recombinant vector, wherein a DNA stated in claim 12 is functionally linked to an expression control sequence.
18. A recombinant vector, wherein a DNA stated in claim 13 is functionally linked to an expression control sequence.
19. A recombinant vector, wherein the DNA stated in claim 14 is functionally linked to an expression control sequence.
20. An animal cell, comprising being transfected simultaneously by a recombinant vector in which a DNA coding for a chimeric protein comprising the  $\alpha$  chain of an integrin and the heavy chain or light chain of an immunoglobulin is functionally linked to an expression control sequence, and a recombinant vector in which a DNA coding for a chimeric protein comprising the  $\beta$  chain of the integrin and the heavy chain or light chain of the immunoglobulin is functionally linked to an expression control sequence.
21. An animal cell, according to claim 20, which is transfected simultaneously by the recombinant vectors stated in claims 16 and 17.
22. An animal cell, according to claim 20, which is transfected simultaneously by the recombinant vectors stated in claims 18 and 19.
23. A method for producing the chimeric protein heterodimer complex stated in claim 2, comprising culturing the animal

cell stated in claim 20.

24. A drug, comprising a chimeric protein or chimeric protein heterodimer complex stated in any one of claims 1 through 9.

25. A drug composition, comprising a chimeric protein or chimeric protein heterodimer complex stated in any one of claims 1 through 9.

26. A platelet substitute, comprising an isolated extracellular matrix receptor as an active ingredient.

27. A platelet substitute, according to claim 26, wherein the extracellular matrix receptor is an integrin.

28. A platelet substitute, according to claim 27, wherein the  $\alpha$  chain of an integrin is  $\alpha 1$ ,  $\alpha 2$ ,  $\alpha 3$ ,  $\alpha 4$ ,  $\alpha 5$ ,  $\alpha 6$ ,  $\alpha 7$ ,  $\alpha 8$ ,  $\alpha 9$ ,  $\alpha v$ ,  $\alpha L$ ,  $\alpha M$ ,  $\alpha X$ ,  $\alpha 11b$  or  $\alpha E$ .

29. A platelet substitute, according to claim 27, wherein the  $\beta$  chain of an integrin is  $\beta 1$ ,  $\beta 2$ ,  $\beta 3$ ,  $\beta 4$ ,  $\beta 5$ ,  $\beta 6$ ,  $\beta 7$  or  $\beta 8$ .

30. A platelet substitute, according to claim 27, wherein the integrin is integrin  $\alpha 2 \beta 1$ .

31. A platelet substitute, according to claim 26, wherein the extracellular matrix receptor is a chimeric protein heterodimer complex comprising an extracellular matrix receptor and an immunoglobulin.

32. A platelet substitute, according to claim 31, wherein the chimeric protein heterodimer complex is a chimeric protein heterodimer complex comprising an integrin and an immunoglobulin.

33. A platelet substitute, according to claim 32, wherein the chimeric protein heterodimer complex is the chimeric protein heterodimer complex stated in claim 2.
34. A platelet substitute, according to claim 33, wherein the chimeric protein heterodimer complex is the chimeric protein heterodimer complex stated in claim 6.
35. A platelet substitute, according to any one of claims 26 through 34, wherein the extracellular matrix receptor is bound to a carrier when used.
36. A platelet substitute, according to any one of claims 26 through 35, which is hemostatic.
37. A method for testing the binding between a chimeric protein heterodimer complex stated in any one of claims 2 to 9, and a ligand or cells, comprising the steps of bringing a chimeric protein heterodimer complex comprising an integrin and an immunoglobulin, and a ligand or cells into contact with each other, to prepare a mixture, and measuring the amount of the chimeric protein heterodimer complex bound to the ligand or cells or the amount of the ligand or cells bound to the chimeric protein heterodimer complex.
38. A method for searching for a substance capable of being bound to an integrin, comprising using a chimeric protein heterodimer complex stated in any one of claims 2 through 9.
39. A substance capable of being bound to an integrin, obtained by using the method stated in claim 38.

40. A method for searching for a substance which inhibits the binding between an integrin and a ligand, comprising using the method stated in claim 37.

41. A method, according to claim 40, wherein the ligand is a fibronectin fragment identified as sequence No. 3 or a collagen.

42. A protein, peptide or low molecular weight compound which inhibits the binding between an integrin and a ligand, obtained by using the method stated in claim 40 or 41.

43. A method for measuring the amount of a ligand of an integrin, comprising using a chimeric protein heterodimer complex stated in any one of claims 2 through 9.

44. A method for identifying an extracellular matrix exposed region, comprising using a chimeric protein heterodimer complex stated in any one of claims 2 through 9.

### Abstract

The present invention provides integrin-immunoglobulin chimeric protein heterodimer complexes in which the  $\alpha$  chain and the  $\beta$  chain of an integrin are stably associated. The obtained integrin-immunoglobulin chimeric protein heterodimer complexes can be directly used as medicines, and can also be used for determining the binding between an integrin and a ligand, and searching for a substance capable of being bound to an integrin and a substance inhibiting the binding between an integrin and a ligand. They can also be used as diagnostic reagents.

Furthermore, it has been found that an integrin isolated with a stably associated structure can be bound to an extracellular matrix under physiological conditions and in the presence of plasma components. Thus, it has been found that an integrin or an extracellular matrix receptor can be applied as a platelet substitute.

# Sequence Table

<110> Kainoh, Mie

Tanaka, Toshiaki

<120> Chimeric proteins, their heterodimer complexes, and platelet substitutes

<130> 1102-98

<141> 1998-09-29

<150> PCT/JP98/00370

<151> 1997-01-29

<160> 31

<170> Wordperfect 5.1

<210> 1

<211> 4228

<212> DNA

<213> Homo sapien

<220>

<221> CDS

<222> 1...2958, 3316...3360, 3480...3808, 3905...4228

<300>

<400> 1

atg	ttc	ccc	acc	gag	agc	gca	tgg	ctt	ggg	aag	cga	ggc	gcg	aac	ccg	48
Met	Phe	Pro	Thr	Glu	Ser	Ala	Trp	Leu	Gly	Lys	Arg	Gly	Ala	Asn	Pro	
				-35					-30					-25		
ggc	ccc	gaa	gct	gca	ctc	cgg	gag	acg	gtg	atg	ctg	ttg	ctg	tgc	ctg	96
Gly	Pro	Glu	Ala	Ala	Leu	Arg	Glu	Thr	Val	Met	Leu	Leu	Leu	Cys	Leu	
			-20					-15					-10			
ggg	gtc	ccg	acc	ggc	agg	cct	tac	aac	gtg	gac	act	gag	agc	gcg	ctg	144
Gly	Val	Pro	Thr	Gly	Arg	Pro	Tyr	Asn	Val	Asp	Thr	Glu	Ser	Ala	Leu	
		-5					1				5					
ctt	tac	cag	ggc	ccc	cac	aac	acg	ctg	ttc	ggc	tac	tcg	gtc	gtg	ctg	192
Leu	Tyr	Gln	Gly	Pro	His	Ans	Thr	Leu	Phe	Gly	Tyr	Ser	Val	Val	Leu	

10					15					20					25	
cac	agc	cac	ggg	gcg	aac	cga	tgg	ctc	cta	gtg	ggt	gcg	ccc	act	gcc	240
His	Ser	His	Gly	Ala	Asn	Arg	Trp	Leu	Leu	Val	Gly	Ala	Pro	Thr	Ala	
				30				35						40		
aac	tgg	ctc	gcc	aac	gct	tca	gtg	atc	aat	ccc	ggg	gcg	att	tac	aga	288
Asn	Trp	Leu	Ala	Asn	Ala	Ser	Val	Ile	Asn	Pro	Gly	Ala	Ile	Tyr	Arg	
			45					50					55			
tgc	acg	atc	gga	aag	aat	ccc	ggc	cag	acg	tgc	gaa	cag	ctc	cag	ctg	336
Cys	Arg	Ile	Gly	Lys	Asn	Pro	Gly	Gln	Thr	Cys	Glu	Gln	Leu	Gln	Leu	
		60					65					70				
ggt	agc	cct	aat	gga	gaa	cct	tgt	gga	aag	act	tgt	ttg	gaa	gag	aga	384
Gly	Ser	Pro	Asn	Gly	Glu	Pro	Cys	Gly	Lys	Thr	Cys	Leu	Glu	Glu	Arg	
	75					80					85					
gac	aat	cag	tgg	ttg	ggg	gtc	aca	ctt	tcc	aga	cag	cca	gga	gaa	aat	432
Asp	Asn	Gln	Trp	Leu	Gly	Val	Thr	Leu	Ser	Arg	Gln	Pro	Gly	Glu	Asn	
90					95					100					105	
gga	tcc	atc	gtg	act	tgt	ggg	cat	aga	tgg	aaa	aat	ata	ttt	tac	ata	480
Gly	Ser	Ile	Val	Thr	Cys	Gly	His	Arg	Trp	Lys	Asn	Ile	Phe	Tyr	Ile	
				110					115					120		
aag	aat	gaa	aat	aag	ctc	ccc	act	ggt	ggt	tgc	tat	gga	gtg	ccc	cct	528
Lys	Asn	Glu	Asn	Lys	Leu	Pro	Thr	Gly	Gly	Cys	Tyr	Gly	Val	Pro	Pro	
			125					130					135			
gat	tta	cga	aca	gaa	ctg	agt	aaa	aga	ata	gct	ccg	tgt	tat	caa	gat	576
Asp	Leu	Arg	Thr	Glu	Leu	Ser	Lys	Arg	Ile	Ala	Pro	Cys	Tyr	Gln	Asp	
		140					145					150				
tat	gtg	aaa	aaa	ttt	gga	gaa	aat	ttt	gca	tca	tgt	caa	gct	gga	ata	624
Tyr	Val	Lys	Lys	Phe	Gly	Glu	Asn	Phe	Ala	Ser	Cys	Gln	Ala	Gly	Ile	
	155					160					165					
tcc	agt	ttt	tac	aca	aag	gat	tta	att	gtg	atg	ggg	gcc	cca	gga	tca	672
Ser	Ser	Phe	Tyr	Thr	Lys	Asp	Leu	Ile	Val	Met	Gly	Ala	Pro	Gly	Ser	
170					175					180					185	
tct	tac	tgg	act	ggc	tct	ctt	ttt	gtc	tac	aat	ata	act	aca	aat	aaa	720
Ser	Tyr	Trp	Thr	Gly	Ser	Leu	Phe	Val	Tyr	Asn	Ile	Thr	Thr	Asn	Lys	
				190					195					200		
tac	aag	gct	ttt	tta	gac	aaa	caa	aat	caa	gta	aaa	ttt	gga	agt	tat	768
Tyr	Lys	Ala	Phe	Leu	Asp	Lys	Gln	Asn	Gln	Val	Lys	Phe	Gly	Ser	Tyr	
		205						210					215			
tta	gga	tat	tca	gtc	gga	gct	ggt	cat	ttt	cgg	agc	cag	cat	act	acc	816
Leu	Gly	Tyr	Ser	Val	Gly	Ala	Gly	His	Phe	Arg	Ser	Gln	His	Thr	Thr	

			220				225				230						
gaa	gta	gtc	gga	gga	gct	cct	caa	cat	gag	cag	att	ggt	aag	gca	tat	864	
Glu	Val	Val	Gly	Gly	Ala	Pro	Gln	His	Glu	Gln	Ile	Gly	Lys	Ala	Try		
			235				240				245						
ata	ttc	agc	att	gat	gaa	aaa	gaa	cta	aat	atc	tta	cat	gaa	atg	aaa	912	
Ile	Phe	Ser	Ile	Asp	Glu	Lys	Glu	Leu	Asn	Ile	Leu	His	Glu	Met	Lys		
			250				255				260				265		
ggt	aaa	aag	ctt	gga	tcg	tac	ttt	gga	gct	tct	gtc	tgt	gct	gtg	gac		
Gly	Lys	Lys	Leu	Gly	Ser	Tyr	Phe	Gly	Ala	Ser	Val	Cys	Ala	Val	Asp		
			270				275				280						
ctc	aat	gca	gat	ggc	ttc	tca	gat	ctg	ctc	gtg	gga	gca	ccc	atg	cag	1008	
Leu	Asn	Ala	Asp	Gly	Phe	Ser	Asp	Leu	Leu	Val	Gly	Ala	Pro	Met	Gln		
			285				290				295						
agc	acc	atc	aga	gag	gaa	gga	aga	gtg	ttt	gtg	tac	atc	aac	tct	ggc	1056	
Ser	Thr	Ile	Arg	Glu	Glu	Gly	Arg	Val	Phe	Val	Try	Ile	Asn	Ser	Gly		
			300				305				310						
tcg	gga	gca	gta	atg	aat	gca	atg	gaa	aca	aac	ctc	gtt	gga	agt	gac	1104	
Ser	Gly	Ala	Val	Met	Asn	Ala	Met	Glu	Thr	Asn	Leu	Val	Gly	Ser	Asp		
			315				320				325						
aaa	tat	gct	gca	aga	ttt	ggg	gaa	tct	ata	gtt	aat	ctt	ggc	gac	att	1152	
Lys	Tyr	Ala	Ala	Arg	Phe	Gly	Glu	Ser	Ile	Val	Asn	Leu	Gly	Asp	Ile		
			330				335				340				345		
gac	aat	gat	ggc	ttt	gaa	gat	gtt	gct	atc	gga	gct	cca	caa	gaa	gat	1200	
Asp	Asn	Asp	Gly	Phe	Glu	Asp	Val	Ala	Ile	Gly	Ala	Pro	Gln	Glu	Asp		
			350				355				360						
gac	ttg	caa	ggt	gct	att	tat	att	tac	aat	ggc	cgt	gca	gat	ggg	atc	1248	
Asp	Leu	Gln	Gly	Ala	Ile	Tyr	Ile	Tyr	Asn	Gly	Arg	Ala	Asp	Gly	Ile		
			365				370				375						
tcg	tca	acc	ttc	tca	cag	aga	att	gaa	gga	ctt	cag	atc	agc	aaa	tcg	1296	
Ser	Ser	Thr	Phe	Ser	Gln	Arg	Ile	Glu	Gly	Leu	Gln	Ile	Ser	Lys	Ser		
			380				385				390						
tta	agt	atg	ttt	gga	cag	tct	ata	tca	gga	caa	att	gat	gca	gat	att	1344	
Leu	Ser	Met	Phe	Gly	Gln	Ser	Ile	Ser	Gly	Gln	Ile	Asp	Ala	Asp	Asn		
			395				400				405						
aat	ggc	tat	gta	gat	gta	gca	ggt	cgt	gct	ttt	cgg	tct	gat	tct	gct	1392	
Asn	Gly	Tyr	Val	Asp	Val	Ala	Val	Gly	Ala	Phe	Arg	Ser	Asp	Ser	Ala		
			410				415				420				425		
gtc	ttg	cta	agg	aca	aga	cct	gta	gta	att	gtt	gac	gct	tct	tta	agc	1440	
Val	Leu	Leu	Arg	Thr	Arg	Pro	Val	Val	Ile	Val	Asp	Ala	Ser	Leu	Ser		

			430					435					440			1488
cac	cct	gag	tca	gta	aat	aga	acg	aaa	ttt	gac	tgt	gtt	gaa	aat	gga	
His	Pro	Glu	Ser	Val	Asn	Arg	Thr	Lys	Phe	Asp	Cys	Val	Glu	Asn	Gly	
			445					450					455			1536
tgg	cct	tct	gtg	tgc	ata	gat	cta	aca	ctt	tgt	ttc	tca	tat	aag	ggc	
Trp	Pro	Ser	Val	Cys	Ile	Asp	Leu	Thr	Leu	Cys	Phe	Ser	Tyr	Lys	Gly	
			460					465					470			1584
aag	gaa	gtt	cca	ggg	tac	att	gtt	ttg	ttt	tat	aac	atg	agt	ttg	gat	
Lys	Glu	Val	Pro	Gly	Tyr	Ile	Val	Leu	Phe	Tyr	Asn	Met	Ser	Leu	Asp	
			475					480					485			1632
gtg	aac	aga	aag	gca	gag	tct	cca	cca	aga	ttc	tat	ttc	tct	tct	aat	
Val	Asn	Arg	Lys	Ala	Glu	Ser	Pro	Pro	Arg	Phe	Tyr	Phe	Ser	Ser	Asn	
			490					495					500			505
gga	act	tct	gac	gtg	att	aca	gga	agc	ata	cag	gtg	tcc	agc	aga	gaa	1680
Gly	Thr	Ser	Asp	Val	Ile	Thr	Gly	Ser	Ile	Gln	Val	Ser	Ser	Arg	Glu	
			510					515					520			1728
gct	aac	tgt	aga	aca	cat	caa	gca	ttt	atg	cgg	aaa	gat	gtg	cgg	gac	
Ala	Asn	Cys	Arg	Thr	His	Gln	Ala	Phe	Met	Arg	Lys	Asp	Val	Arg	Asp	
			525					530					535			1776
atc	ctc	acc	cca	att	cag	att	gaa	gct	gct	tac	cac	ctt	ggg	cct	cat	
Ile	Leu	Thr	Pro	Ile	Gln	Ile	Glu	Ala	Ala	Tyr	His	Leu	Gly	Pro	His	
			540					545					550			1824
gtc	atc	agt	aaa	cga	agt	aca	gag	gaa	ttc	cca	cca	ctt	cag	cca	att	
Val	Ile	Ser	Lys	Arg	Ser	Thr	Glu	Glu	Phe	Pro	Pro	Leu	Gln	Pro	Ile	
			555					560					565			1872
ctt	cag	cag	aag	aaa	gaa	aaa	gac	ata	atg	aaa	aaa	aca	ata	aac	ttt	
Leu	Gln	Gln	Lys	Lys	Glu	Lys	Asp	Ile	Met	Lys	Lys	Thr	Ile	Asn	Phe	
			570					575					580			585
gca	agg	ttt	tgt	gcc	cat	gaa	aat	tgt	tct	gct	gat	tta	cag	gtt	tct	1920
Ala	Arg	Phe	Cys	Ala	His	Glu	Asn	Cys	Ser	Ala	Asp	Leu	Gln	Val	Ser	
			590					595					600			1968
gca	aag	att	ggg	ttt	ttg	aag	ccc	cat	gaa	aat	aaa	aca	tat	ctt	gct	
Ala	Lys	Ile	Gly	Phe	Leu	Lys	Pro	His	Glu	Asn	Lys	Thr	Tyr	Leu	Ala	
			605					610					615			2016
gtt	ggg	agt	atg	aag	aca	ttg	atg	ttg	aat	gtg	tcc	ttg	ttt	aat	gct	
Val	Gly	Ser	Met	Lys	Thr	Leu	Met	Leu	Asn	Val	Ser	Leu	Phe	Asn	Ala	
			620					625					630			2064
gga	gat	gat	gca	tat	gaa	acg	act	cta	cat	gtc	aaa	cta	ccc	gtg	ggg	
Gly	Asp	Asp	Ala	Tyr	Glu	Thr	Thr	Leu	His	Val	Lys	Leu	Pro	Val	Gly	

	635					640					645					
ctt	tat	ttc	att	aag	att	tta	gag	ctg	gaa	gag	aag	caa	ata	aac	tgt	2112
Leu	Tyr	Phe	Ile	Lys	Ile	Leu	Glu	Leu	Glu	Glu	Lys	Gln	Ile	Asn	Cys	
650						655					660				665	
gaa	gtc	aca	gat	aac	tct	ggc	gtg	gta	caa	ctt	gac	tgc	agt	att	ggc	2160
Glu	Val	Thr	Asp	Asn	Ser	Gly	Val	Val	Gln	Leu	Asp	Cys	Ser	Ile	Gly	
				670					675						680	
tat	ata	tat	gta	gat	cat	ctc	tca	agg	ata	gat	att	agc	ttt	ctc	ctg	2208
Tyr	Ile	Tyr	Val	Asp	His	Leu	Ser	Arg	Ile	Asp	Ile	Ser	Phe	Leu	Leu	
			685					690					695			
gat	gtg	agc	tca	ctc	agc	aga	gcg	gaa	gag	gac	ctc	agt	atc	aca	gtg	2256
Asp	Val	Ser	Ser	Leu	Ser	Arg	Ala	Glu	Glu	Asp	Leu	Ser	Ile	Thr	Val	
		700					705					710				
cat	gct	acc	tgt	gaa	aat	gaa	gag	gaa	atg	gac	aat	cta	aag	cac	agc	2304
His	Ala	Thr	Cys	Glu	Asn	Glu	Glu	Glu	Met	Asp	Asn	Leu	Lys	His	Ser	
	715					720					725					
aga	gtg	act	gta	gca	ata	cct	tta	aaa	tat	gag	gtt	aag	ctg	act	gtt	2352
Arg	Val	Thr	Val	Ala	Ile	Pro	Leu	Lys	Tyr	Glu	Val	Lys	Leu	Thr	Val	
730						735				740					745	
cat	ggg	ttt	gta	aac	cca	act	tca	ttt	gtg	tat	gga	tca	aat	gat	gaa	2400
His	Gly	Phe	Val	Asn	Pro	Thr	Ser	Phe	Val	Tyr	Gly	Ser	Asn	Asp	Glu	
				750				755						760		
aat	gag	cct	gaa	acg	tgc	atg	gtg	gag	aaa	atg	aac	tta	act	ttc	cat	2448
Asn	Glu	Pro	Glu	Thr	Cys	Met	Val	Glu	Lys	Met	Asn	Leu	Thr	Phe	His	
		765						770				775				
gtt	atc	aac	act	ggc	aat	agt	atg	gct	ccc	aat	gtt	agt	gtg	gaa	ata	2496
Val	Ile	Asn	Thr	Gly	Asn	Ser	Met	Ala	Pro	Asn	Val	Ser	Val	Glu	Ile	
		780					785					790				
atg	gta	cca	aat	tct	ttt	agc	ccc	caa	act	gat	aag	ctg	ttc	aac	att	2588
Met	Val	Pro	Asn	Ser	Phe	Ser	Pro	Gln	Thr	Asp	Lys	Leu	Phe	Asn	Ile	
	795					800					805					
ttg	gat	gtc	cag	act	act	act	gga	gaa	tgc	cac	ttt	gaa	aat	tat	caa	2592
Leu	Asp	Val	Gln	Thr	Thr	Thr	Gly	Glu	Cys	His	Phe	Glu	Asn	Tyr	Gln	
810						815				820					825	
aga	gtg	tgt	gca	tta	gag	cag	caa	aag	agt	gca	atg	cag	acc	ttg	aaa	2640
Arg	Val	Cys	Ala	Leu	Glu	Gln	Gln	Lys	Ser	Ala	Met	Gln	Thr	Leu	Lys	
				830				835						840		
ggc	ata	gtc	cgg	ttc	ttg	tcc	aag	act	gat	aag	agg	cta	ttg	tac	tgc	2688
Gly	Ile	Val	Arg	Phe	Leu	Ser	Lys	Thr	Asp	Lys	Arg	Leu	Leu	Tyr	Cys	

		845						850				855					
ata	aaa	gct	gat	cca	cat	tgt	tta	aat	ttc	ttg	tgt	aat	ttt	ggg	aaa	2736	
lle	Lys	Ala	Asp	Pro	His	Cys	Leu	Asn	Phe	Leu	Cys	Asn	Phe	Gly	Lys		
		860						865				870					
atg	gaa	agt	gga	aaa	gaa	gcc	agt	gtt	cat	atc	caa	ctg	gaa	ggc	cgg	2784	
Met	Glu	Ser	Gly	Lys	Glu	Ala	Ser	Val	His	Ile	Gln	Leu	Glu	Gly	Arg		
		875						880				885					
cca	tcc	att	tta	gaa	atg	gat	gag	act	tca	gca	ctc	aag	ttt	gaa	ata	2832	
Pro	Ser	Ile	Leu	Glu	Met	Asp	Glu	Thr	Ser	Ala	Leu	Lys	Phe	Glu	Ile		
		890						895				900				905	
aga	gca	aca	ggg	ttt	cca	gag	cca	aat	cca	aga	gta	att	gaa	cta	aac	2880	
Arg	Ala	Thr	Gly	Phe	Pro	Glu	Pro	Asn	Pro	Arg	Vai	Ile	Glu	Leu	Asn		
		910						915				920					
aag	gat	gag	aat	gtt	gcg	cat	gtt	cta	ctg	gaa	gga	cta	cat	cat	caa	2928	
Lys	Asp	Glu	Asn	Val	Ala	His	Val	Leu	Leu	Glu	Gly	Leu	His	His	Gln		
		925						930				935					
aga	ccc	aaa	cgt	tat	ttc	acg	gat	ccc	gag	ctgctggaag			caggctcagc			2978	
Arg	Pro	Lys	Arg	Tyr	Phe	Thr	Asp	Pro	Glu								
		940						945									
gctcctgcct		ggacgcattcc		cggctatgca		gccccagtc		agggcagcaa		ggcaggcccc						3038	
gtctgcctct		tcaccggag		cctctgcccg		ccccactcat		gctcaggag		agggctctct						3098	
ggctttttcc		caggctctgg		gcaggcacag		gctaggtgcc		cctaaccag		gccctgcaca						3158	
caaaggggca		gggtctgggc		tcagacctgc		caagagccat		atccgggagg		accctgcccc						3218	
tgacctaaagc		ccaccccaaa		ggccaaactc		tccactccct		cagctcggac		accttctctc						3278	
ctcccagatt		ccagtaactc		ccaatcttct		ctctgca		gag		ccc	aaa	tct	tgt	gac	3333		
								Glu		Pro	Lys	Ser	Cys	Asp			
								950									
aaa	act	cac	aca	tgc	cca	ccg	tgc	cca	ggtaagccag		cccaggcctc		3380				
Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro									
		955						960									
gccccccagc		tcaaggcggg		acagggtccc		tagagtagcc		tgcattccagg		gacaggcccc		3440					
agccggggtgc		tgacacgtcc		acctccatct		cttctctca		gca		cct	gaa	ctc	ctg	3493			
								Ala		Pro	Glu	Leu	Leu				
								965									
ggg	gga	ccg	tca	gtc	ttc	ctc	ttc	ccc	cca	aaa	ccc	aag	gac	acc	ctc	3541	
Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu		
		970						975				980					
atg	atc	tcc	cgg	acc	cct	gag	gtc	aca	tgc	gtg	gtg	gtg	gac	gtg	agc	3589	
Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser		

	985					990					995						
cac	gaa	gac	cct	gag	gtc	aag	ttc	aac	tgg	tac	gtg	gac	ggc	gtg	gag	3637	
His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu		
1000					1005					1010					1015		
gtg	cat	aat	gcc	aag	aca	aag	ccg	cgg	gag	gag	cag	tac	aac	agc	acg	3685	
Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr		
1020					1025					1030							
tac	cgg	gtg	gtc	agc	gtc	ctc	acc	gtc	ctg	cac	cag	gac	tgg	ctg	aat	3733	
Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn		
1035					1040					1045							
ggc	aag	gag	tac	aag	tgc	aag	gtc	tcc	aac	aaa	gcc	ctc	cca	gcc	ccc	3781	
Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro		
1050					1055					1060							
atc	gag	aaa	acc	atc	tcc	aaa	gcc	aaa	ggtgggacc	gtgggggtgcg						3828	
Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys									
1065					1070												
agggccacat		ggacagaggc		cggctcggcc		caccctctgc		cctgagagtg		accgctgtac						3888	
caacctctgt		cctaca		ggg		cag	ccc	cga	gaa	cca	cag	gtg	tac	acc	ctg	3937	
					Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu		
					1075					1080							
ccc	cca	tcc	cgg	gat	gag	ctg	acc	aag	aac	cag	gtc	agc	ctg	acc	tgc	3985	
Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys		
1085					1090					1095							
ctg	gtc	aaa	ggc	ttc	tat	ccc	agc	gac	atc	gcc	gtg	gag	tgg	gag	agc	4033	
Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser		
1100					1105					1110					1115		
aat	ggg	cag	ccg	gag	aac	aac	tac	aag	acc	acg	cct	ccc	gtg	ctg	gat	4081	
Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp		
					1120					1125					1130		
tcc	gac	ggc	tcc	ttc	ttc	ctc	tac	agc	aag	ctc	acc	gtg	gac	aag	agc	4129	
Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser		
					1135					1140					1145		
agg	tgg	cag	cag	ggg	aac	gtc	ttc	tca	tgc	tcc	gtg	atg	cat	gag	gct	4177	
Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala		
1150					1155					1160							
ctg	cac	aac	cac	tac	acg	cag	aag	agc	ctc	tcc	ctg	tct	ccg	ggt	aaa	4225	
Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys		
1165					1170					1175							
tga																4228	

<210> 2

<211> 3463

<212> DNA

<213> Homo sapien

<220>

<221> CDS

<222> 1...2193, 2551...2595, 2714...3043, 3140...3463

<300>

<400> 2

atg	aat	tta	caa	cca	att	ttc	tgg	att	gga	ctg	atc	agt	tca	gtt	tgc	48
Met	Asn	Leu	Gln	Pro	Ile	Phe	Trp	Ile	Gly	Leu	Ile	Ser	Ser	Val	Cys	
-20					-15					-10					-5	
tgt	gtg	ttt	gct	caa	aca	gat	gaa	aat	aga	tgt	tta	aaa	gca	aat	gcc	96
Cys	Val	Phe	Ala	Gln	Thr	Asp	Glu	Asn	Arg	Cys	Leu	Lys	Ala	Asn	Ala	
				1				5					10			
aaa	tca	tgt	gga	gaa	tgt	ata	caa	gca	ggg	cca	aat	tgt	ggg	tgg	tgc	144
Lys	Ser	Cys	Gly	Glu	Cys	Ile	Gln	Ala	Gly	Pro	Asn	Cys	Gly	Trp	Cys	
		15					20					25				
aca	aat	tca	aca	ttt	tta	cag	gaa	gga	atg	cct	act	tct	gca	cga	tgt	192
Thr	Asn	Ser	Thr	Phe	Leu	Gln	Glu	Gly	Met	Pro	Thr	Ser	Ala	Arg	Cys	
	30					35					40					
gat	gat	tta	gaa	gcc	tta	aaa	aag	aag	ggt	tgc	cct	cca	gat	gac	ata	240
Asp	Asp	Leu	Glu	Ala	Leu	Lys	Lys	Lys	Gly	Cys	Pro	Pro	Asp	Asp	Ile	
45					50					55					60	
gaa	aat	ccc	aga	ggc	tcc	aaa	gat	ata	aag	aaa	aat	aaa	aat	gta	acc	288
Glu	Asn	Pro	Arg	Gly	Ser	Lys	Asp	Ile	Lys	Lys	Asn	Lys	Asn	Val	Thr	
				65					70					75		
aac	cgt	agc	aaa	gga	aca	gca	gag	aag	ctc	aag	cca	gag	gat	att	cat	336
Asn	Arg	Ser	Lys	Gly	Thr	Ala	Glu	Lys	Leu	Lys	Pro	Glu	Asp	Ile	His	
			80					85					90			
cag	atc	caa	cca	cag	cag	ttg	gtt	ttg	cga	tta	aga	tca	ggg	gag	cca	384
Gln	Ile	Gln	Pro	Gln	Gln	Leu	Val	Leu	Arg	Leu	Arg	Ser	Gly	Glu	Pro	
		95					100					105				
cag	aca	ttt	aca	tta	aaa	ttc	aag	aga	gct	gaa	gac	tat	ccc	att	gac	432
Gln	Thr	Phe	Thr	Leu	Lys	Phe	Lys	Arg	Ala	Glu	Asp	Tyr	Pro	Ile	Asp	

	110					115					120					
ctc	tac	tac	ctt	atg	gac	ctg	tct	tat	tca	atg	aaa	gac	gat	ttg	gag	480
Leu	Tyr	Tyr	Leu	Met	Asp	Leu	Ser	Tyr	Ser	Met	Lys	Asp	Asp	Leu	Glu	
125					130					135					140	
aat	gta	aaa	agt	ctt	gga	aca	gat	ctg	atg	aat	gaa	atg	agg	agg	att	528
Asn	Val	Lys	Ser	Leu	Gly	Thr	Asp	Leu	Met	Asn	Glu	Met	Arg	Arg	Ile	
				145					150					155		
act	tcg	gac	ttc	aga	att	gga	ttt	ggc	tca	ttt	gtg	gaa	aag	act	gtg	576
Thr	Ser	Asp	Phe	Arg	Ile	Gly	Phe	Gly	Ser	Phe	Val	Glu	Lys	Thr	Val	
			160					165					170			
atg	cct	tac	att	agc	aca	aca	cca	gct	aag	ctc	agg	aac	cct	tgc	aca	624
Met	Pro	Tyr	Ile	Ser	Thr	Thr	Pro	Ala	Lys	Leu	Arg	Asn	Pro	Cys	Thr	
		175					180					185				
agt	gaa	cag	aac	tgc	acc	acc	cca	ttt	agc	tac	aaa	aat	gtg	ctc	agt	672
Ser	Glu	Gln	Asn	Cys	Thr	Thr	Pro	Phe	Ser	Tyr	Lys	Asn	Val	Leu	Ser	
	190					195					200					
ctt	act	aat	aaa	gga	gaa	gta	ttt	aat	gaa	ctt	gtt	gga	aaa	cag	cgc	720
Leu	Thr	Asn	Lys	Gly	Glu	Val	Phe	Asn	Glu	Leu	Val	Gly	Lys	Gln	Arg	
205					210					215					220	
ata	tct	gga	aat	ttg	gat	tct	cca	gaa	ggt	ggt	ttc	gat	gcc	atc	atg	768
Ile	Ser	Gly	Asn	Leu	Asp	Ser	Pro	Glu	Gly	Gly	Phe	Asp	Ala	Ile	Met	
				225					230					235		
caa	gtt	gca	gtt	tgt	gga	tca	ctg	att	ggc	tgg	agg	aat	gtt	aca	cgg	816
Gln	Val	Ala	Val	Cys	Gly	Ser	Leu	Ile	Gly	Trp	Arg	Asn	Val	Thr	Arg	
			240					245					250			
ctg	ctg	gtg	ttt	tcc	aca	gat	gcc	ggg	ttt	cac	ttt	gct	gga	gat	ggg	864
Leu	Leu	Val	Phe	Ser	Thr	Asp	Ala	Gly	Phe	His	Phe	Ala	Gly	Asp	Gly	
		255					260					265				
aaa	ctt	ggt	ggc	att	gtt	tta	cca	aat	gat	gga	caa	tgt	cac	ctg	gaa	912
Lys	Leu	Gly	Gly	Ile	Val	Leu	Pro	Asn	Asp	Gly	Gln	Cys	His	Leu	Glu	
	270					275				280						
aat	aat	atg	tac	aca	atg	agc	cat	tat	tat	gat	tat	cct	tct	att	gct	960
Asn	Asn	Met	Tyr	Thr	Met	Ser	His	Tyr	Tyr	Asp	Tyr	Pro	Ser	Ile	Ala	
285					290					295					300	
cac	ctt	gtc	cag	aaa	ctg	agt	gaa	aat	aat	att	cag	aca	att	ttt	gca	1008
His	Leu	Val	Gln	Lys	Leu	Ser	Glu	Asn	Asn	Ile	Gln	Thr	Ile	Phe	Ala	
			305					310						315		
gtt	act	gaa	gaa	ttt	cag	cct	gtt	tac	aag	gag	ctg	aaa	aac	ttg	atc	1056
Val	Thr	Glu	Glu	Phe	Gln	Pro	Val	Tyr	Lys	Glu	Leu	Lys	Asn	Leu	Ile	

cct	aag	tca	gca	gta	gga	aca	tta	tct	gca	aat	tct	agc	aat	gta	att	1104
Pro	Lys	Ser	Ala	Val	Gly	Thr	Leu	Ser	Ala	Asn	Ser	Ser	Asn	Val	Ile	
		335						340					345			
cag	ttg	atc	att	gat	gca	tac	att	tcc	ctt	tcc	tca	gaa	gtc	att	ttg	1152
Gln	Leu	Ile	Ile	Asp	Ala	Tyr	Asn	Ser	Leu	Ser	Ser	Glu	Val	Ile	Leu	
		350						355					360			
gaa	aac	ggc	aaa	ttg	tca	gaa	gga	gta	aca	ata	agt	tac	aaa	tct	tac	1200
Glu	Asn	Gly	Lys	Leu	Ser	Glu	Gly	Val	Thr	Ile	Ser	Tyr	Lys	Ser	Tyr	
365																
tgc	aag	aac	ggg	gtg	aat	gga	aca	ggg	gaa	aat	gga	aga	aaa	tgt	tcc	1248
Cys	Lys	Asn	Gly	Val	Asn	Gly	Thr	Gly	Glu	Asn	Gly	Arg	Lys	Cys	Ser	
				385												
aat	att	tcc	att	gga	gat	gag	gtt	caa	ttt	gaa	att	agc	ata	act	tca	1296
Asn	Ile	Ser	Ile	Gly	Asp	Glu	Val	Gln	Phe	Glu	Ile	Ser	Ile	Thr	Ser	
			400													
aat	aag	tgt	cca	aaa	aag	gat	tct	gac	agc	ttt	aaa	att	agg	cct	ctg	1344
Asn	Lys	Cys	Pro	Lys	Lys	Asp	Ser	Asp	Ser	Phe	Lys	Ile	Arg	Pro	Leu	
			415													
ggc	ttt	acg	gag	gaa	gta	gag	gtt	att	ctt	cag	tac	atc	tgt	gaa	tgt	1392
Gly	Phe	Thr	Glu	Glu	Val	Glu	Val	Ile	Leu	Gln	Tyr	Ile	Cys	Glu	Cys	
gaa	tgc	caa	agc	gaa	ggc	atc	cct	gaa	agt	ccc	aag	tgt	cat	gaa	gga	1440
Glu	Cys	Gln	Ser	Glu	Gly	Ile	Pro	Glu	Ser	Pro	Lys	Cys	His	Glu	Gly	
445																
aat	ggg	aca	ttt	gag	tgt	ggc	gcg	tgc	agg	tgc	aat	gaa	ggg	cgt	gtt	1488
Asn	Gly	Thr	Phe	Glu	Cys	Gly	Ala	Cys	Arg	Cys	Asn	Glu	Gly	Arg	Val	
				465												
ggt	aga	cat	tgt	gaa	tgc	agc	aca	gat	gaa	gtt	aac	agt	gaa	gac	atg	1536
Gly	Arg	His	Cys	Glu	Cys	Ser	Thr	Asp	Glu	Val	Asn	Ser	Glu	Asp	Met	
			480													
gat	gct	tac	tgc	agg	aaa	gaa	aac	agt	tca	gaa	atc	tgc	agt	aac	aat	1584
Asp	Ala	Tyr	Cys	Arg	Lys	Glu	Asn	Ser	Ser	Glu	Ile	Cys	Ser	Asn	Asn	
			495													
gga	gag	tgc	gtc	tgc	gga	cag	tgt	gtt	tgt	agg	aag	agg	gat	aat	aca	1632
Gly	Glu	Cys	Val	Cys	Gly	Gln	Cys	Val	Cys	Arg	Lys	Arg	Asp	Asn	Thr	
aat	gaa	att	tat	tct	ggc	aaa	ttc	tgc	gag	tgt	gat	aat	ttc	aac	tgt	1680
Asn	Glu	Ile	Tyr	Ser	Gly	Lys	Phe	Cys	Glu	Cys	Asp	Asn	Phe	Asn	Cys	

525					530					535					540				
gat	aga	tcc	aat	ggc	tta	att	tgt	gga	gga	aat	ggg	gtt	tgc	aag	tgt	1728			
Asp	Arg	Ser	Asn	Gly	Leu	Ile	Cys	Gly	Gly	Asn	Gly	Val	Cys	Lys	Cys				
				545					550					555					
cgt	gtg	tgt	gag	tgc	aac	ccc	aac	tac	act	ggc	agt	gca	tgt	gac	tgt	1776			
Arg	Val	Cys	Glu	Cys	Asn	Pro	Asn	Tyr	Thr	Gly	Ser	Ala	Cys	Asp	Cys				
				560					565					570					
tct	ttg	gat	act	agt	act	tgt	gaa	gcc	agc	aac	gga	cag	atc	tgc	aat	1824			
Ser	Leu	Asp	Thr	Ser	Thr	Cys	Glu	Ala	Ser	Asn	Gly	Gln	Ile	Cys	Asn				
				575					580					585					
ggc	cgg	ggc	atc	tgc	gag	tgt	ggg	gtc	tgt	aag	tgt	aca	gat	ccg	aag	1872			
Gly	Arg	Gly	Ile	Cys	Glu	Cys	Gly	Val	Cys	Lys	Cys	Thr	Asp	Pro	Lys				
				590					595					600					
ttt	caa	ggg	caa	acg	tgt	gag	atg	tgt	cag	acc	tgc	ctt	ggg	gtc	tgt	1920			
Phe	Gln	Gly	Gln	Thr	Cys	Glu	Met	Cys	Gln	Thr	Cys	Leu	Gly	Val	Cys				
				605					610					615					
gct	gag	cat	aaa	gaa	tgt	gtt	cag	tgc	aga	gcc	ttc	aat	aaa	gga	gaa	1968			
Ala	Glu	His	Lys	Glu	Cys	Val	Gln	Cys	Arg	Ala	Phe	Asn	Lys	Gly	Glu				
				625					630					635					
aag	aaa	gac	aca	tgc	aca	cag	gaa	tgt	tcc	tat	ttt	aac	att	acc	aag	2016			
Lys	Lys	Asp	Thr	Cys	Thr	Gln	Glu	Cys	Ser	Tyr	Phe	Asn	Ile	Thr	Lys				
				640					645					650					
gta	gaa	agt	cgg	gac	aaa	tta	ccc	cag	ccg	gtc	caa	cct	gat	cct	gtg	2064			
Val	Glu	Ser	Arg	Asp	Lys	Leu	Pro	Gln	Pro	Val	Gln	Pro	Asp	Pro	Val				
				655					660					665					
tcc	cat	tgt	aag	gag	aag	gat	gtt	gac	gac	tgt	tgg	ttc	tat	ttt	acg	2112			
Ser	His	Cys	Lys	Glu	Lys	Asp	Val	Asp	Asp	Cys	Trp	Phe	Tyr	Phe	Thr				
				670					675					680					
tat	tca	gtg	aat	ggg	aac	aac	gag	gtc	atg	gtt	cat	gtt	gtg	gag	aat	2160			
Tyr	Ser	Val	Asn	Gly	Asn	Asn	Glu	Val	Met	Val	His	Val	Val	Glu	Asn				
				685					690					695					
cca	gag	tgt	ccc	act	ggg	cca	gag	gat	ccc	gag	ctgctggaag				caggctcagc		2213		
Pro	Glu	Cys	Pro	Thr	Gly	Pro	Glu	Asp	Pro	Glu									
				705					710										
gctcctgcct			ggacgcattc			cggctatgca			gccccagttc			agggcagcaa			ggcaggccccc			2273	
gtctgcctct			tcacccggag			cctctgcccg			ccccactcat			gctcagggag			aggggtcttct			2333	
ggcctttttcc			caggctctgg			gcaggcacag			gctaggtgcc			cctaaccacag			gccctgcaca			2393	
caaagggggca			gggtgctgggc			tcagacctgc			caagagccat			atccggggagg			accctgcccc			2453	
tgacctaaagc			ccacccccaaa			ggccaaaactc			tccactccct			cagctcggac			accttctctc			2513	

ctcccagatt			ccagtaactc			ccaatcttct			ctctgca		gag		ccc	aaa	tct	tgt	gac	2568
											Glu	Pro	Lys	Ser	Cys	Asp		
													715					
aaa	act	cac	aca	tgc	cca	ccg	tgc	cca	ggtaagccag			cccagggcctc						2615
Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro										
			720						725									
gccctccagc			tcaaggcggg			acaggtgccc			tagagtagcc			tgcattccagg			gacaggcccc			2675
agccggggtgc			tgacacgtcc			acctccatct			cttcctca			gca	cct	gaa	ctc	ctg		2728
												Ala	Pro	Glu	Leu	Leu		
															730			
ggg	gga	ccg	tca	gtc	ttc	ctc	ttc	ccc	cca	aaa	ccc	aag	gac	acc	ctc		2776	
Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu			
			735						740						745			
atg	atc	tcc	cgg	acc	cct	gag	gtc	aca	tgc	gtg	gtg	gtg	gac	gtg	agc		2824	
Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser			
			750						755						760			
cac	gaa	gac	cct	gag	gtc	aag	ttc	aac	tgg	tac	gtg	gac	ggc	gtg	gag		2872	
His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu			
			765						770			775						
gtg	cat	aat	gcc	aag	aca	aag	ccg	cgg	gag	gag	cag	tac	aac	agc	acg		2920	
Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr			
780						785						790						795
tac	cgg	gtg	gtc	agc	gtc	ctc	acc	gtc	ctg	cac	cag	gac	tgg	ctg	aat		2968	
Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn			
			800						805						810			
ggc	aag	gag	tac	aag	tgc	aag	gtc	tcc	aac	aaa	gcc	ctc	cca	gcc	ccc		3016	
Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro			
			815						820						825			
atc	gag	aaa	acc	atc	tcc	aaa	gcc	aaa	ggtagggacc			gtgggggtgcg						3063
Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys										
			830						835									
agggccacat			ggacagaggc			cggtcggcc			caccctctgc			cctgagagtg			accgctgtac			3123
caacctctgt			cctaca			ggg	cag	ccc	cga	gaa	cca	cag	gtg	tac	acc	ctg		3172
						Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu		
									840						845			
ccc	cca	tcc	cgg	gat	gag	ctg	acc	aag	aac	cag	gtc	agc	ctg	acc	tgc		3220	
Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys			
			850						855						860			
ctg	gtc	aaa	ggc	ttc	tat	ccc	agc	gac	atc	gcc	gtg	gag	tgg	gag	agc		3268	

Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	
	865					870					875					
aat	ggg	cag	ccg	gag	aac	aac	tac	aag	acc	acg	cct	ccc	gtg	ctg	gat	3316
Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	
880					885					890					895	
tcc	gac	ggc	tcc	ttc	ttc	ctc	tac	agc	aag	ctc	acc	gtg	gac	aag	agc	3364
Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	
				900					905					910		
agg	tgg	cag	cag	ggg	aac	gtc	ttc	tca	tgc	tcc	gtg	atg	cat	gag	gct	3412
Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	
			915					920					925			
ctg	cac	aac	cac	tac	acg	cag	aag	agc	ctc	tcc	ctg	tct	ccg	ggg	aaa	3460
Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys	
		930					935					940				
tga																3463

<210> 3

<211> 13

<212> PRT

<213> Artificial Sequence

<220> Peptide

<223> Synthetic peptide

Linear

<400> 3

Cys	Leu	His	Gly	Pro	Glu	Ile	Leu	Asp	Val	Pro	Ser	Thr
1				5					10			

<210> 4

<211> 31

<212> DNA

<213> Artificial Sequence

<220>

<221> Synthetic DNA

<223> Oligonucleotide

20250404 14:53:53

<400> 4

gcggatcccg agctgctgga agcaggctca g

31

<210> 5

<211> 27

<212> DNA

<213> Artificial Sequence

<220>

<221> Synthetic DNA

<223> Oligonucleotide

<400> 5

cctctagacg gccgtcgac tcattta

27

<210> 6

<211> 73

<212> DNA

<213> Artificial Sequence

<220>

<221> Synthetic DNA

<223> Oligonucleotide

<400> 6

ctagaccacc atgttcccca ccgagagcgc atggcttggg aagcgaggcg cgaacccggg  
ccccggagct gca

73

<210> 7

<211> 65

<212> DNA

<213> Artificial Sequence

<220>

<221> Synthetic DNA

<223> Oligonucleotide

<400> 7

gcttcggggc      ccgggttcgc      gcctcgcttc      ccaagccatg      cgctctcggt      ggggaacatg  
gtggt

65

<210> 8

<211> 51

<212> DNA

<213> Artificial Sequence

<220>

<221> Synthetic DNA

<223> Oligonucleotide

<400> 8

ctccgggaga      cggatgatgct      gttgctgtgc      ctgggggtcc      cgaccggcag      g

51

<210> 9

<211> 55

<212> DNA

<213> Artificial Sequence

<220>

<221> Synthetic DNA

<223> Oligonucleotide

<400> 9

cctgccggtc      gggaccccca      ggcacagcaa      cagcatcacc      gtctcccga      gtcga

55

<210> 10

<211> 37

<212> DNA

<213> Artificial Sequence

<220>

<221> Synthetic DNA

<223> Oligonucleotide

<400> 10

cactgcaggc aggccttaca acgtggacac tgagagc

37

<210> 11

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<221> Synthetic DNA

<223> Oligonucleotide

<400> 11

gcagaaacct gtaaatcagc ag

22

<210> 12

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<221> Synthetic DNA

<223> Oligonucleotide

<400> 12

gcatttatgc ggaaagatgt gc

22

<210> 13

<211> 29

<212> DNA

<213> Artificial Sequence

<220>

<221> Synthetic DNA

<223> Oligonucleotide

<400> 13

cgggatccgt      gaaataacgt      ttgggtctt

29

<210> 14

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<221> Synthetic DNA

<223> Oligonucleotide

<400> 14

gcggaaaaga      tgaattaca      ac

22

<210> 15

<211> 27

<212> DNA

<213> Artificial Sequence

<220>

<221> Synthetic DNA

<223> Oligonucleotide

<400> 15

gtgggatcct      ctggaccagt      gggacac

27

<210> 16

<211> 10

<212> PRT

<213> Artificial Sequence

<220> Peptide

<223> Synthetic peptide

Linear

<400> 16

Gly	Pro	Glu	Ile	Leu	Asp	Val	Pro	Ser	Thr
1				5					10

<210> 17

<211> 10

<212> PRT

<213> Artificial Sequence

<220> Peptide

<223> Synthetic peptide

Linear

<400> 17

Gly	Pro	Glu	Ile	Leu	Glu	Val	Pro	Ser	Thr
1				5					10

<210> 18

<211> 6

<212> PRT

<213> Artificial Sequence

<220> Peptide

<223> Synthetic Peptide

Linear

<400> 18

Gly	Arg	Gly	Asp	Ser	Pro
1				5	

<210> 19

<211> 4675

<212> DNA

<213> Homo sapien

<220>

615 616 617 618 619 620 621 622 623 624 625 626 627 628 629 630 631 632 633 634 635 636 637 638 639 640 641 642 643 644 645 646 647 648 649 650 651 652 653 654 655 656 657 658 659 660 661 662 663 664 665 666 667 668 669 670 671 672 673 674 675 676 677 678 679 680 681 682 683 684 685 686 687 688 689 690 691 692 693 694 695 696 697 698 699 700 701 702 703 704 705 706 707 708 709 710 711 712 713 714 715 716 717 718 719 720 721 722 723 724 725 726 727 728 729 730 731 732 733 734 735 736 737 738 739 740 741 742 743 744 745 746 747 748 749 750 751 752 753 754 755 756 757 758 759 760 761 762 763 764 765 766 767 768 769 770 771 772 773 774 775 776 777 778 779 780 781 782 783 784 785 786 787 788 789 790 791 792 793 794 795 796 797 798 799 800 801 802 803 804 805 806 807 808 809 810 811 812 813 814 815 816 817 818 819 820 821 822 823 824 825 826 827 828 829 830 831 832 833 834 835 836 837 838 839 840 841 842 843 844 845 846 847 848 849 850 851 852 853 854 855 856 857 858 859 860 861 862 863 864 865 866 867 868 869 870 871 872 873 874 875 876 877 878 879 880 881 882 883 884 885 886 887 888 889 890 891 892 893 894 895 896 897 898 899 900 901 902 903 904 905 906 907 908 909 910 911 912 913 914 915 916 917 918 919 920 921 922 923 924 925 926 927 928 929 930 931 932 933 934 935 936 937 938 939 940 941 942 943 944 945 946 947 948 949 950 951 952 953 954 955 956 957 958 959 960 961 962 963 964 965 966 967 968 969 970 971 972 973 974 975 976 977 978 979 980 981 982 983 984 985 986 987 988 989 990 991 992 993 994 995 996 997 998 999 1000

<221> CDS

<222> 1...3405, 3763...3807, 3926...4255, 4352...4675

<300>

<400> 19

atg	ggg	cca	gaa	cgg	aca	ggg	gcc	gcg	ccg	ctg	ccg	ctg	ctg	ctg	gtg	48
Met	Gly	Pro	Glu	Arg	Thr	Gly	Ala	Ala	Pro	Leu	Pro	Leu	Leu	Leu	Val	
				-25					-20						-15	
tta	gcg	ctc	agt	caa	ggc	att	tta	aat	tgt	tgt	ttg	gcc	tac	aat	gtt	96
Leu	Ala	Leu	Ser	Gln	Gly	Ile	Leu	Asn	Cys	Cys	Leu	Ala	Tyr	Asn	Val	
			-10					-5					1			
ggg	ctc	cca	gaa	gca	aaa	ata	ttt	tcc	ggg	cct	tca	agt	gaa	cag	ttt	114
Gly	Leu	Pro	Glu	Ala	Lys	Ile	Phe	Ser	Gly	Pro	Ser	Ser	Glu	Gln	Phe	
	5					10					15					
ggg	tat	gca	gtg	cag	cag	ttt	ata	aat	cca	aaa	ggc	aac	tgg	tta	ctg	192
Gly	Tyr	Ala	Val	Gln	Gln	Phe	Ile	Asn	Pro	Lys	Gly	Asn	Trp	Leu	Leu	
	20				25					30					35	
gtt	ggg	tca	ccc	tgg	agt	ggc	ttt	cct	gag	aac	cga	atg	gga	gat	gtg	240
Val	Gly	Ser	Pro	Trp	Ser	Gly	Phe	Pro	Glu	Asn	Arg	Met	Gly	Asp	Val	
				40					45					50		
tat	aaa	tgt	cct	gtt	gac	cta	tcc	act	gcc	aca	tgt	gaa	aaa	cta	aat	288
Tyr	Lys	Cys	Pro	Val	Asp	Leu	Ser	Thr	Ala	Thr	Cys	Glu	Lys	Leu	Asn	
			55					60					65			
ttg	caa	act	tca	aca	agc	att	cca	aat	gtt	act	gag	atg	aaa	acc	aac	336
Leu	Gln	Thr	Ser	Thr	Ser	Ile	Pro	Asn	Val	Thr	Glu	Met	Lys	Thr	Asn	
		70					75					80				
atg	agc	ctc	ggc	ttg	atc	ctc	acc	agg	aac	atg	gga	act	gga	ggg	ttt	384
Met	Ser	Leu	Gly	Leu	Ile	Leu	Thr	Arg	Asn	Met	Gly	Thr	Gly	Gly	Phe	
	85					90					95					
ctc	aca	tgt	ggg	cct	ctg	tgg	gca	cag	caa	tgt	ggg	aat	cag	tat	tac	432
Leu	Thr	Cys	Gly	Pro	Leu	Trp	Ala	Gln	Gln	Cys	Gly	Asn	Gln	Tyr	Tyr	
	100					105				110					115	
aca	acg	ggg	gtg	tgt	tct	gac	atc	agt	cct	gat	ttt	cag	ctc	tca	gcc	480
Thr	Thr	Gly	Val	Cys	Ser	Asp	Ile	Ser	Pro	Asp	Phe	Gln	Leu	Ser	Ala	
				120					125					130		
agc	ttc	tca	cct	gca	act	cag	ccc	tgc	cct	tcc	ctc	ata	gat	gtt	gtg	528
Ser	Phe	Ser	Pro	Ala	Thr	Gln	Pro	Cys	Pro	Ser	Leu	Ile	Asp	Val	Val	
			135					140					145			
gtt	gtg	tgt	gat	gaa	tca	aat	agt	att	tat	cct	tgg	gat	gca	gta	aag	576
Val	Val	Cys	Asp	Glu	Ser	Asn	Ser	Ile	Tyr	Pro	Trp	Asp	Ala	Val	Lys	
		150					155					160				
aat	ttt	ttg	gaa	aaa	ttt	gta	caa	ggc	ctt	gat	ata	ggc	ccc	aca	aag	624

Asn	Phe	Leu	Glu	Lys	Phe	Val	Gln	Gly	Leu	Asp	Ile	Gly	Pro	Thr	Lys	
	165					170					175					
aca	cag	gtg	ggg	tta	att	cag	tat	gcc	aat	aat	cca	aga	ggt	gtg	ttt	672
Thr	Gln	Val	Gly	Leu	Ile	Gln	Tyr	Ala	Asn	Asn	Pro	Arg	Val	Val	Phe	
180					185					190					195	
aac	ttg	aac	aca	tat	aaa	acc	aaa	gaa	gaa	atg	att	gta	gca	aca	tcc	720
Asn	Leu	Asn	Thr	Tyr	Lys	Thr	Lys	Glu	Glu	Met	Ile	Val	Ala	Thr	Ser	
				200					205					210		
cag	aca	tcc	caa	tat	ggg	ggg	gac	ctc	aca	aac	aca	ttc	gga	gca	att	768
Gln	Thr	Ser	Gln	Tyr	Gly	Gly	Asp	Leu	Thr	Asn	Thr	Phe	Gly	Ala	Ile	
			215					220					225			
caa	tat	gca	aga	aaa	tat	gcc	tat	tca	gca	gct	tct	ggg	ggg	cga	cga	816
Gln	Tyr	Ala	Arg	Lys	Tyr	Ala	Tyr	Ser	Ala	Ala	Ser	Gly	Gly	Arg	Arg	
		230					235					240				
agt	gct	acg	aaa	gta	atg	gta	ggt	gta	act	gac	ggg	gaa	tca	cat	gat	864
Ser	Ala	Thr	Lys	Val	Met	Val	Val	Val	Thr	Asp	Gly	Glu	Ser	His	Asp	
	245					250					255					
ggg	tca	atg	ttg	aaa	gct	gtg	att	gat	caa	tgc	aac	cat	gac	aat	ata	912
Gly	Ser	Met	Leu	Lys	Ala	Val	Ile	Asp	Gln	Cys	Asn	His	Asp	Asn	Ile	
260					265					270					275	
ctg	agg	ttt	ggc	ata	gca	gtt	ctt	ggg	tac	tta	aac	aga	aac	gcc	ctt	960
Leu	Arg	Phe	Gly	Ile	Ala	Val	Leu	Gly	Tyr	Leu	Asn	Arg	Asn	Ala	Leu	
				280					285					290		
gat	act	aaa	aat	tta	ata	aaa	gaa	ata	aaa	gcg	atc	gct	agt	att	cca	1008
Asp	Thr	Lys	Asn	Leu	Ile	Lys	Glu	Ile	Lys	Ala	Ile	Ala	Ser	Ile	Pro	
		295						300					305			
aca	gaa	aga	tac	ttt	ttc	aat	gtg	tct	gat	gaa	gca	gct	cta	cta	gaa	1056
Thr	Glu	Arg	Tyr	Phe	Phe	Asn	Val	Ser	Asp	Glu	Ala	Ala	Leu	Leu	Glu	
		310					315					320				
aag	gct	ggg	aca	tta	gga	gaa	caa	att	ttc	agc	att	gaa	ggg	act	gtt	1104
Lys	Ala	Gly	Thr	Leu	Gly	Glu	Gln	Ile	Phe	Ser	Ile	Glu	Gly	Thr	Val	
	325					330					335					
caa	gga	gga	gac	aac	ttt	cag	atg	gaa	atg	tca	caa	gtg	gga	ttc	agt	1152
Gln	Gly	Gly	Asp	Asn	Phe	Gln	Met	Glu	Met	Ser	Gln	Val	Gly	Phe	Ser	
340					345					350					355	
gca	gat	tac	tct	tct	caa	aat	gat	att	ctg	atg	ctg	ggg	gca	gtg	gga	1200
Ala	Asp	Tyr	Ser	Ser	Gln	Asn	Asp	Ile	Leu	Met	Leu	Gly	Ala	Val	Gly	
				360					365					370		
gct	ttt	ggc	tgg	agt	ggg	acc	att	gtc	cag	aag	aca	tct	cat	ggc	cat	1248
Ala	Phe	Gly	Trp	Ser	Gly	Thr	Ile	Val	Gln	Lys	Thr	Ser	His	Gly	His	
			375					380					385			
ttg	atc	ttt	cct	aaa	caa	gcc	ttt	gac	caa	att	ctg	cag	gac	aga	aat	1296



Phe	Gly	Gln	Val	Val	Gln	Leu	Trp	Ser	Gln	Ser	Ile	Ala	Asp	Val	Ala	
			615					620					625			
ata	gaa	gct	tca	ttc	aca	cca	gaa	aaa	atc	act	ttg	gtc	aac	aag	aat	2016
Ile	Glu	Ala	Ser	Phe	Thr	Pro	Glu	Lys	Ile	Thr	Leu	Val	Asn	Lys	Asn	
		630					635					640				
gct	cag	ata	att	ctc	aaa	ctc	tgc	ttc	agt	gca	aag	ttc	aga	cct	act	2064
Ala	Gln	Ile	Ile	Leu	Lys	Leu	Cys	Phe	Ser	Ala	Lys	Phe	Arg	Pro	Thr	
		645				650					655					
aag	caa	aac	aat	caa	gtg	gcc	att	gta	tat	aac	atc	aca	ctt	gat	gca	2112
Lys	Gln	Asn	Asn	Gln	Val	Ala	Ile	Val	Tyr	Asn	Ile	Thr	Leu	Asp	Ala	
		660			665					670					675	
gat	gga	ttt	tca	tcc	aga	gta	acc	tcc	agg	ggg	tta	ttt	aaa	gaa	aac	2160
Asp	Gly	Phe	Ser	Ser	Arg	Val	Thr	Ser	Arg	Gly	Leu	Phe	Lys	Glu	Asn	
			680						685						690	
aat	gaa	agg	tgc	ctg	cag	aag	aat	atg	gta	gta	aat	caa	gca	cag	agt	2208
Asn	Glu	Arg	Cys	Leu	Gln	Lys	Asn	Met	Val	Val	Asn	Gln	Ala	Gln	Ser	
			695					700					705			
tgc	ccc	gag	cac	atc	att	tat	ata	cag	gag	ccc	tct	gat	gtt	gtc	aac	2256
Cys	Pro	Glu	His	Ile	Ile	Tyr	Ile	Gln	Glu	Pro	Ser	Asp	Val	Val	Asn	
		710					715					720				
tct	ttg	gat	ttg	cgt	gtg	gac	atc	agt	ctg	gaa	aac	cct	ggc	act	agc	2304
Ser	Leu	Asp	Leu	Arg	Val	Asp	Ile	Ser	Leu	Glu	Asn	Pro	Gly	Thr	Ser	
		725				730					735					
cct	gcc	ctt	gaa	gcc	tat	tct	gag	act	gcc	aag	gtc	ttc	agt	att	cct	2352
Pro	Ala	Leu	Glu	Ala	Tyr	Ser	Glu	Thr	Ala	Lys	Val	Phe	Ser	Ile	Pro	
		740			745					750					755	
ttc	cac	aaa	gac	tgt	ggg	gag	gat	gga	ctt	tgc	att	tct	gat	cta	gtc	2400
Phe	His	Lys	Asp	Cys	Gly	Glu	Asp	Gly	Leu	Cys	Ile	Ser	Asp	Leu	Val	
			760					765							770	
cta	gat	gtc	cga	caa	ata	cca	gct	gct	caa	gaa	caa	ccc	ttt	att	gtc	2448
Leu	Asp	Val	Arg	Gln	Ile	Pro	Ala	Ala	Gln	Glu	Gln	Pro	Phe	Ile	Val	
			775					780					785			
agc	aac	caa	aac	aaa	agg	tta	aca	ttt	tca	gta	aca	ctg	aaa	aat	aaa	2496
Ser	Asn	Gln	Asn	Lys	Arg	Leu	Thr	Phe	Ser	Val	Thr	Leu	Lys	Asn	Lys	
		790					795					800				
agg	gaa	agt	gca	tac	aac	act	gga	att	gtt	gtt	gat	ttt	tca	gaa	aac	2544
Arg	Glu	Ser	Ala	Tyr	Asn	Thr	Gly	Ile	Val	Val	Asp	Phe	Ser	Glu	Asn	
		805				810					815					
ttg	ttt	ttt	gca	tca	ttc	tcc	cta	ccg	gtt	gat	ggg	aca	gaa	gta	aca	2592
Leu	Phe	Phe	Ala	Ser	Phe	Ser	Leu	Pro	Val	Asp	Gly	Thr	Glu	Val	Thr	
					825					830					835	
820																
tgc	cag	gtg	gct	gca	tct	cag	aag	tct	gtt	gcc	tgc	gat	gta	ggc	tac	2640

Cys	Gln	Val	Ala	Ala	Ser	Gln	Lys	Ser	Val	Ala	Cys	Asp	Val	Gly	Tyr	
				840					845					850		
cct	gct	tta	aag	aga	gaa	caa	cag	gtg	act	ttt	act	att	aac	ttt	gac	2688
Pro	Ala	Leu	Lys	Arg	Glu	Gln	Gln	Val	Thr	Phe	Thr	Ile	Asn	Phe	Asp	
			855					860					865			
ttc	aat	ctt	caa	aac	ctt	cag	aat	cag	gcg	tct	ctc	agt	ttc	caa	gcc	2736
Phe	Asn	Leu	Gln	Asn	Leu	Gln	Asn	Gln	Ala	Ser	Leu	Ser	Phe	Gln	Ala	
			870				875					880				
tta	agt	gaa	agc	caa	gaa	gaa	aac	aag	gct	gat	aat	ttg	gtc	aac	ctc	2784
Leu	Ser	Glu	Ser	Gln	Glu	Glu	Asn	Lys	Ala	Asp	Asn	Leu	Val	Asn	Leu	
	885					890					895					
aaa	att	cct	ctc	ctg	tat	gat	gct	gaa	att	cac	tta	aca	aga	tct	acc	2832
Lys	Ile	Pro	Leu	Leu	Tyr	Asp	Ala	Glu	Ile	His	Leu	Thr	Arg	Ser	Thr	
					905					910					915	
aac	ata	aat	ttt	tat	gaa	atc	tct	tcg	gat	ggg	aat	gtt	cct	tca	atc	2880
Asn	Ile	Asn	Phe	Tyr	Glu	Ile	Ser	Ser	Asp	Gly	Asn	Val	Pro	Ser	Ile	
				920					925					930		
gtg	cac	agt	ttt	gaa	gat	gtt	ggg	cca	aaa	ttc	atc	ttc	tcc	ctg	aag	2928
Val	His	Ser	Phe	Glu	Asp	Val	Gly	Pro	Lys	Phe	Ile	Phe	Ser	Leu	Lys	
			935					940					945			
gta	aca	aca	gga	agt	gtt	cca	gta	agc	atg	gca	act	gta	atc	atc	cac	2976
Val	Thr	Thr	Gly	Ser	Val	Pro	Val	Ser	Met	Ala	Thr	Val	Ile	Ile	His	
			950				955					960				
atc	cct	cag	tat	acc	aaa	gaa	aag	aac	cca	ctg	atg	tac	cta	act	ggg	3024
Ile	Pro	Gln	Tyr	Thr	Lys	Glu	Lys	Asn	Pro	Leu	Met	Tyr	Leu	Thr	Gly	
	965					970					975					
gtg	caa	aca	gac	aag	gct	ggg	gac	atc	agt	tgt	aat	gca	gat	atc	aat	3072
Val	Gln	Thr	Asp	Lys	Ala	Gly	Asp	Ile	Ser	Cys	Asn	Ala	Asp	Ile	Asn	
					985					990					995	
cca	ctg	aaa	ata	gga	caa	aca	tct	tct	tct	gta	tct	ttc	aaa	agt	gaa	3120
Pro	Leu	Lys	Ile	Gly	Gln	Thr	Ser	Ser	Ser	Val	Ser	Phe	Lys	Ser	Glu	
				1000					1005					1010		
aat	ttc	agg	cac	acc	aaa	gaa	ttg	aac	tgc	aga	act	gct	tcc	tgt	agt	3168
Asn	Phe	Arg	His	Thr	Lys	Glu	Leu	Asn	Cys	Arg	Thr	Ala	Ser	Cys	Ser	
			1015					1020					1025			
aat	gtt	acc	tgc	tgg	ttg	aaa	gac	gtt	cac	atg	aaa	gga	gaa	tac	ttt	3216
Asn	Val	Thr	Cys	Trp	Leu	Lys	Asp	Val	His	Met	Lys	Gly	Glu	Tyr	Phe	
		1030					1035					1040				
gtt	aat	gtg	act	acc	aga	att	tgg	aac	ggg	act	ttc	gca	tca	tca	acg	3264
Val	Asn	Val	Thr	Thr	Arg	Ile	Trp	Asn	Gly	Thr	Phe	Ala	Ser	Ser	Thr	
	1045					1050					1055					
ttc	cag	aca	gta	cag	cta	acg	gca	gct	gca	gaa	atc	aac	acc	tat	aac	3312

Phe	Gln	Thr	Val	Gln	Leu	Thr	Ala	Ala	Ala	Glu	Ile	Asn	Thr	Tyr	Asn				
1060					1065					1070					1075				
cct	gag	ata	tat	gtg	att	gaa	gat	aac	act	gtt	acg	att	ccc	ctg	atg	3360			
Pro	Glu	Ile	Tyr	Val	Ile	Glu	Asp	Asn	Thr	Val	Thr	Ile	Pro	Leu	Met				
1080					1085					1090					1095				
ata	atg	aaa	cct	gat	gag	aaa	gcc	gaa	gta	cca	aca	gat	ccc	gag		3405			
Ile	Met	Lys	Pro	Asp	Glu	Lys	Ala	Glu	Val	Pro	Thr	Asp	Pro	Glu					
1095					1100					1105					1110				
ctgctggaag		caggctcagc		gctcctgcct		ggacgcattcc		cggtatgca		gccccagtc							3465		
agggcagcaa		ggcaggccccc		gtctgcctct		tcaccggag		cctctgcccg		ccccactcat							3525		
gctcaggggag		agggctcttct		ggctttttcc		caggctctgg		gcaggcacag		gctaggtgcc							3585		
cctaaccag		gccctgcaca		caaaggggca		ggctgctgggc		tcagacctgc		caagagccat							3645		
atccgggagg		accctgcccc		tgacctagc		ccaccccaaa		ggccaaactc		tccactccct							3705		
cagctcggac		accttctctc		ctcccagatt		ccagtaactc		ccaatcttct		ctctgca							3762		
gag	ccc	aaa	tct	tgt	gac	aaa	act	cac	aca	tgc	cca	ccg	tgc	cca		3807			
Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro					
1110					1115					1120					1125				
ggtaagccag		cccaggcctc		gccccccagc		tcaaggcggg		acagggtccc		tagagtagcc							3867		
tgcatccagg		gacaggccccc		agccggggtgc		tgacacgtcc		acctccatct		cttctctca							3925		
gca	cct	gaa	ctc	ctg	ggg	gga	ccg	tca	gtc	ttc	ctc	ttc	ccc	cca	aaa	3973			
Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys				
1125					1130					1135					1140				
ccc	aag	gac	acc	ctc	atg	atc	tcc	cgg	acc	cct	gag	gtc	aca	tgc	gtg	4021			
Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val				
1140					1145					1150					1155				
gtg	gtg	gac	gtg	agc	cac	gaa	gac	cct	gag	gtc	aag	ttc	aac	tgg	tac	4069			
Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr				
1155					1160					1165					1170				
gtg	gac	ggc	gtg	gag	gtg	cat	aat	gcc	aag	aca	aag	ccg	cgg	gag	gag	4117			
Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu				
1170					1175					1180					1185				
cag	tac	aac	agc	acg	tac	cgg	gtg	gtc	agc	gtc	ctc	acc	gtc	ctg	cac	4165			
Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His				
1190					1195					1200					1205				
cag	gac	tgg	ctg	aat	ggc	aag	gag	tac	aag	tgc	aag	gtc	tcc	aac	aaa	4213			
Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys				
1205					1210					1215					1220				
gcc	ctc	cca	gcc	ccc	atc	gag	aaa	acc	atc	tcc	aaa	gcc	aaa			4255			
Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys						
1220					1225					1230					1235				
ggtgggaccc		gtgggggtgcg		agggccacat		ggacagaggc		cggtctggcc		caccctctgc							4315		
cctgagagtg		accgctgtac		caacctctgt		cctaca		ggg		cag		ccc		cga	gaa	cca	4369		

[illegible]

<211> 27

<213> Artificial Sequence

<221> Synthetic DNA

<223> Oligonucleotide

<400> 20

27

<210> 21

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<221> Synthetic DNA

<223> Oligonucleotide

<400> 21

atagtgcct gatgaccatt g

21

<210> 22

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<221> Synthetic DNA

<223> Oligonucleotide

<400> 22

gatggcttta atgatgtgat tg

22

<210> 23

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<221> Synthetic DNA

<223> Oligonucleotide

<400> 23

tgttggtact tcggctttct c

21

<210> 24

<211> 8

<212> PRT

<213> Artificial Sequence

<220> Peptide

<223> Synthetic peptide

Circular

<400> 24

Cys	Ile	Pro	Glu	Leu	Ile	Val	Cys
1				5			

<210> 25

<211> 8

<212> PRT

<213> Artificial Sequence

<220> Peptide

<223> Synthetic peptide

Circular

<400> 25

Cys	Met	Arg	Tyr	Thr	Ser	Ala	Cys
1				5			

<210> 26

<211> 8

<212> PRT

<213> Artificial Sequence

<220> Peptide

<223> Synthetic peptide

Circular

<400> 26

Cys	Glu	Trp	Met	Lys	Arg	Phe	Cys
1				5			

<210> 27

<211> 8

<212> PRT

<213> Artificial Sequence

<220> Peptide

<223> Synthetic peptide

Circular

<400> 27

Cys	Tyr	Thr	Thr	Arg	Leu	Lys	Cys
1				5			

<210> 28

<211> 8

<212> PRT

<213> Artificial Sequence

<220> Peptide

<223> Synthetic peptide

Circular

<400> 28

Cys	Leu	Arg	Tyr	Ser	Val	Pro	Cys
1				5			

<210> 29

<211> 8

<212> PRT

<213> Artificial Sequence

<220> Peptide

<223> Synthetic peptide

Circular

<400> 29

Cys	Ile	Val	Asn	Arg	Leu	Gly	Cys
1				5			

<210> 30

<211> 8

<212> PRT

<213> Artificial Sequence

<220> Peptide

<223> Synthetic peptide

Circular

<400> 30

Cys	Gly	Leu	Gln	Ala	Leu	Pro	Cys
1				5			

<210> 31

<211> 8

<212> PRT

<213> Artificial Sequence

<220> Peptide

<223> Synthetic peptide

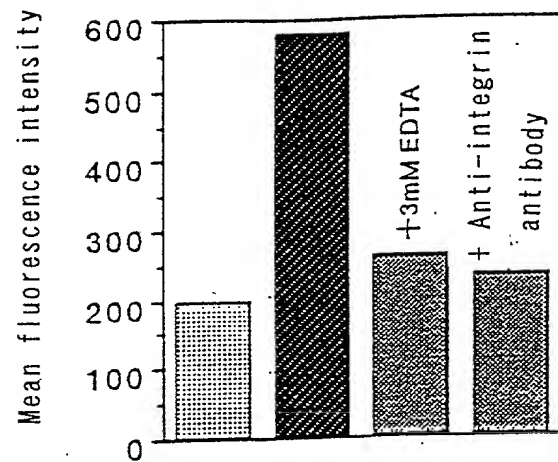
Circular

<400> 31

Cys	Lys	Leu	Lys	Gly	Thr	Met	Cys
1				5			

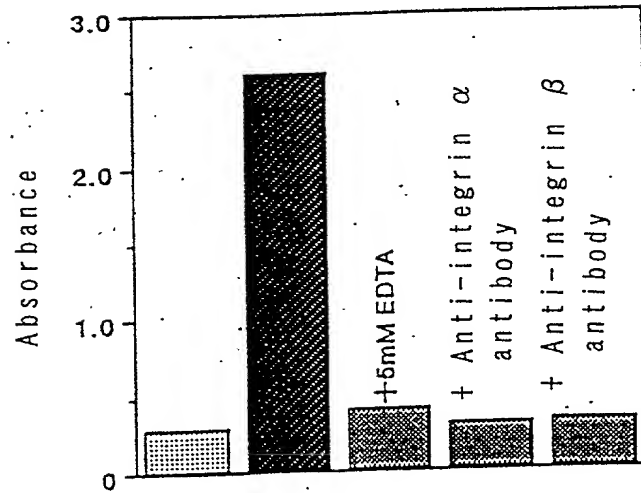
## Drawing

Fig. 1



$\alpha 4 \cdot \text{IgG} - \beta 1 \cdot \text{IgG}$  chimeric protein heterodimer complex (-) (+) (+) (+)

Fig. 2



$\alpha 4 \cdot \text{IgG} - \beta 1 \cdot \text{IgG}$  chimeric protein heterodimer complex (-) (+) (+) (+) (+)

Fig. 3

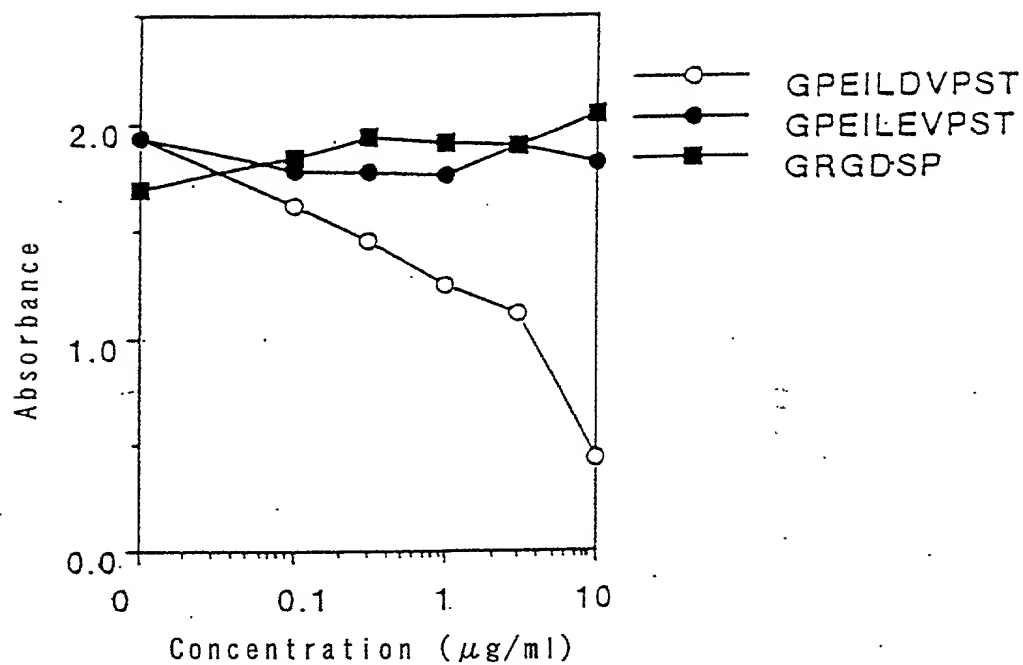


Fig. 4

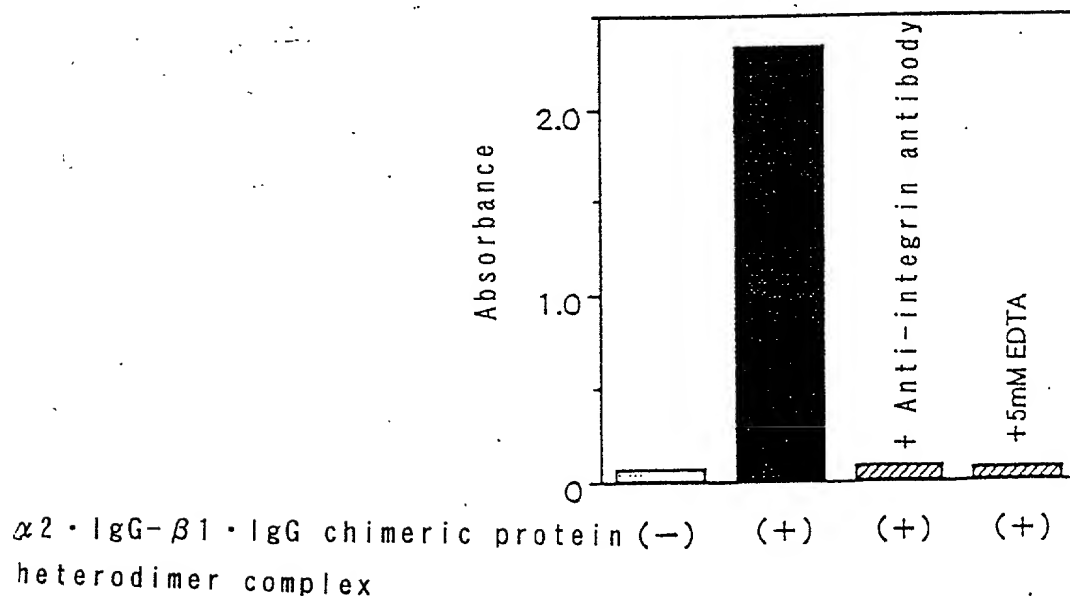
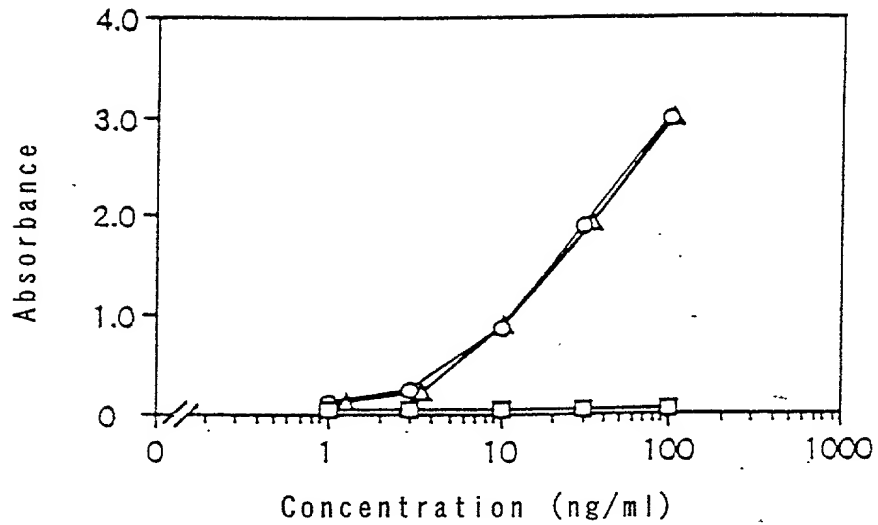
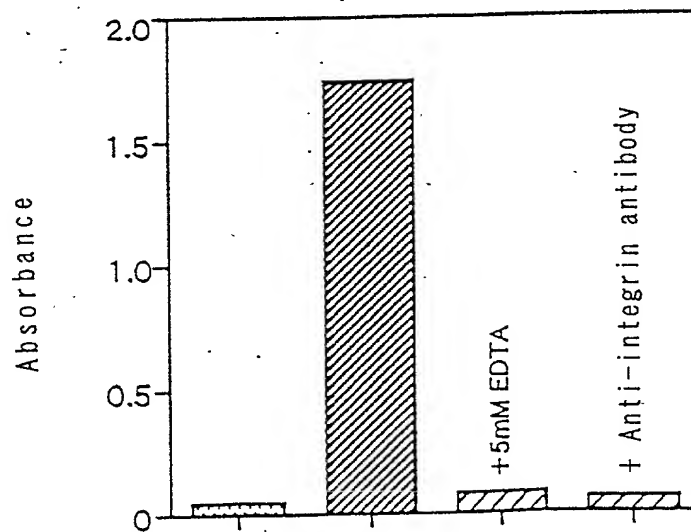


Fig. 5



- Human IgG liposome (normal plasma)
- Integrin  $\alpha 2 \beta 1$  IgG liposome (normal plasma)
- △— Integrin  $\alpha 2 \beta 1$  IgG liposome (von Willebrand's factor deficient plasma)

Fig. 6



$\alpha 2 \cdot \text{IgG} - \beta 1 \cdot \text{IgG}$  chimeric protein (-) (+) (+) (+)  
heterodimer complex liposome

- ☐ Original Application  
☒ PCT National Application  
U.S. Designated Office  
☐ Continuation or Divisional Application  
☐ Continuation-in-Part Application

**COMBINED DECLARATION,  
POWER OF ATTORNEY AND PETITION**

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled CHIMERIC PROTEINS, THEIR HETERODIMER COMPLEXES, AND PLATELET SUBSTITUTES

- ☐ which is described in the specification and claims

☐ attached hereto.

☐ filed on \_\_\_\_\_

Application Serial No. \_\_\_\_\_

and was amended on \_\_\_\_\_

*(if applicable)*

- ☒ which is described in International Application No. PCT/JP98/00370

filed \_\_\_\_\_ and as amended on \_\_\_\_\_

\_\_\_\_\_  
(if any),

which I have reviewed and for which I solicit a United States patent.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I do not know and do not believe that this invention was ever known or used in the United States before my or our invention thereof or patented or described in any printed publication in any country before my or our invention thereof or more than one year prior to this application or said international application, or in public use or on sale in the United States of America more than one year prior to this application or said international application, or that the invention has been patented or made the subject of an inventor's certificate issued before the date of this application or said international application in any country foreign to the United States of America on an application filed by me or my legal representatives or assigns more than twelve months prior to this application or said international application, or that any application for patent or inventor's certificate on this invention has been filed in any country foreign to the United States of America prior to this application or said international application by me or my legal representatives or assigns except as identified below.

**COMBINED DECLARATION, POWER OF ATTORNEY AND PETITION**  
(Page 2)

Attorney Docket No. 1102-98

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign applications for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)

Number	Country	Date of Filing (day, month, year)	Priority Claimed
9-15118	Japan	29 Jan. 1997	<input checked="" type="checkbox"/> yes <input type="checkbox"/> no
9-234544	Japan	29 Aug. 1997	<input checked="" type="checkbox"/> yes <input type="checkbox"/> no
			<input type="checkbox"/> yes <input type="checkbox"/> no
			<input type="checkbox"/> yes <input type="checkbox"/> no
			<input type="checkbox"/> yes <input type="checkbox"/> no

I hereby claim the benefit under Title 35, United States Code, §119 or §120 (as applicable) of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

_____ (Application Serial No.)	_____ (Filing Date)	_____ (Status) (patented, pending, abandoned)
-----------------------------------	------------------------	--

_____ (Application Serial No.)	_____ (Filing Date)	_____ (Status) (patented, pending, abandoned)
-----------------------------------	------------------------	--

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorneys to prosecute this application and transact all business in the United States Patent and Trademark Office connected therewith:

Austin R. Miller	Reg. No. <u>16,602</u>
T. Daniel Christenbury	Reg. No. <u>31,750</u>
Frank A. Cona	Reg. No. <u>38,412</u>
David A. Sasso	Reg. No. <u>43,084</u>
Patrick J. Farley	Reg. No. <u>42,524</u>

SEND CORRESPONDENCE TO:  
Schnader Harrison Segal & Lewis  
36th Floor  
1600 Market Street  
Philadelphia, PA 19103

DIRECT TELEPHONE CALLS TO  
ATTORNEY INDICATED ON PAPER:  
Austin R. Miller  
T. Daniel Christenbury  
Frank A. Cona  
David A. Sasso  
Patrick J. Farley  
(215) 563-1810

**COMBINED DECLARATION, POWER OF ATTORNEY AND PETITION**  
(Page 3)

Attorney Docket No. 1102-98

I hereby petition for grant of a United States Letters Patent on this invention.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1-0 1. FULL NAME OF SOLE OR FIRST INVENTOR <b>Mie Kainoh</b>		INVENTOR'S SIGNATURE <i>Mie Kainoh</i>	DATE <b>Nov. 4 1998</b>
RESIDENCE <b>Kanagawa, Japan</b> <i>SPX</i>		CITIZENSHIP <b>Japan</b>	
POST OFFICE ADDRESS <b>633-1-201 Fujisawa, Fujisawa-shi, Kanagawa 251 Japan</b>			
2-0 2. FULL NAME OF JOINT INVENTOR, IF ANY <b>Toshiaki Tanaka</b>		INVENTOR'S SIGNATURE <i>Toshiaki Tanaka</i>	DATE <b>Nov. 4, 1998</b>
RESIDENCE <b>Kanagawa, Japan</b> <i>SPX</i>		CITIZENSHIP <b>Japan</b>	
POST OFFICE ADDRESS <b>11-24 Numama 1-chome, Zushi-shi, Kanagawa 249 Japan</b>			
3 FULL NAME OF ADDITIONAL JOINT INVENTOR, IF ANY		INVENTOR'S SIGNATURE	DATE
RESIDENCE		CITIZENSHIP	
POST OFFICE ADDRESS			
4 FULL NAME OF ADDITIONAL JOINT INVENTOR, IF ANY		INVENTOR'S SIGNATURE	DATE
RESIDENCE		CITIZENSHIP	
POST OFFICE ADDRESS			
5 FULL NAME OF ADDITIONAL JOINT INVENTOR, IF ANY		INVENTOR'S SIGNATURE	DATE
RESIDENCE		CITIZENSHIP	
POST OFFICE ADDRESS			
6 FULL NAME OF ADDITIONAL JOINT INVENTOR, IF ANY		INVENTOR'S SIGNATURE	DATE
RESIDENCE		CITIZENSHIP	
POST OFFICE ADDRESS			
7 FULL NAME OF ADDITIONAL JOINT INVENTOR, IF ANY		INVENTOR'S SIGNATURE	DATE
RESIDENCE		CITIZENSHIP	
POST OFFICE ADDRESS			